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Madness, responsibility and the law

The law is necessarily an awkward compromise between the principles of justice and the needs of society. This simple truth has been illustrated all too vividly in the past few weeks by yet another sensational murder trial, in the genre that appears to recur every so often in England, and which on this occasion has led to the imprisonment of a Mr Peter Sutcliffe for a period of thirty years, after his admission that he had murdered no fewer than thirteen women over a period of several years. Mr Sutcliffe, when arrested, had confessed to his series of crimes but, at his trial, pleaded not guilty to the charge of murder, on the grounds of "diminished responsibility", but guilty of the lesser charge of manslaughter. The court declined to accept this version of events. The trial (before a jury) was an opportunity both for a rehearsal of the sombre details of Mr Sutcliffe's particular line in the technique of murder and for an examination of his state of mind at the time. Psychiatric specialists who had examined him told the court that Mr Sutcliffe suffered from "paranoid schizophrenia", and for their pains were required to spend several hours in the witness box at the Old Bailey speculating on the question whether Mr Sutcliffe could have learned his schizophrenic symptoms from his wife. In the end, the jury found Mr Sutcliffe guilty, the judge in the case called his crimes "cowardly" and Mr Sutcliffe was sent off to gaol.

The importance of the Sutcliffe case extends beyond what appears to have been its immediate function — providing British society with a cathartic ending to the long and incompetent hunt for a notorious murderer and satisfying the curiosity of the prurient. There are legal questions of peculiarly British interest about the validity of pleas of guilty to a lesser charge on the grounds of diminished responsibility. On this occasion, the British Attorney-General, as the country's principal law officer, had been willing to accept this plea so as to spare the relatives of the murdered women the pain of sordid publicity. (Now that capital punishment has been abolished, convictions for murder and manslaughter carry similar penalties, so that accepting Mr Sutcliffe's plea did not entail a promise of leniency.) In the event, the court declined to accept the compromise and the trial centred on the question of whether Mr Sutcliffe's responsibility for his actions had been undermined by his psychiatric condition. There are plainly enough intricacies in this decision to keep lawyers' dinner tables animated for years to come. The more important and durable issues have not so far been taken up.

The most immediate difficulty is not legal but social and medical. Mr Sutcliffe's case for supposing that he was not fully responsible for his awful actions was that he had the symptoms of paranoid schizophrenia, in particular the hallucination of hearing voices. The chief issue at the trial was whether Mr Sutcliffe might have simulated this and other possible symptoms of schizophrenia. The prosecution, the defence and even the court appear to have taken it as self-evident that if the diagnosis were correct, Mr Sutcliffe's bizarre actions would be accounted for and the plea of diminished responsibility made acceptable.

The fallacy in this line of argument is clear. People who suffer from schizophrenia are unhappily all too common, while paranoia is a common but not invariable symptom. Especially since the introduction of neuroleptic drugs as a palliative for schizophrenia, people diagnosed as paranoid schizophrenics have been living in the community, leading lives often no more aberrant than those considered as within the normal range for people free from psychiatric illness. Mr Sutcliffe had not sought treatment for his supposed illness (although, according to

evidence during the trial, his wife had been treated for schizophrenia). Even so, there is no evidence to suggest that schizophrenia accompanied by paranoid behaviour predisposes towards sexual violence nor even that schizophrenia is more commonly represented than other types of psychotic illness in its causation.

All this goes some way to justify the court's view that the plea of diminished responsibility was unacceptable; evidence of paranoid schizophrenia, the argument seems to have been, is not by itself a proof of a diminished sense of responsibility. The hope now must be that all the talk there has been of the connection between schizophrenia and the likelihood of ending up in the dock at the Old Bailey will not too much unsettle the large numbers of people suffering from this illness who daily face crises less spectacular than Mr Sutcliffe's which are nevertheless potentially as catastrophic. The professional psychiatrists giving evidence last month, in their zeal to do the best for Mr Sutcliffe, may have overlooked the side-effects of what they were saying.

The deeper question is that of the relationship between psychiatric illness as now recognized in modern society and what used to be called madness. Where capital punishment has been done away with, there has usually been an intermediate phase in which the penalties of conviction for capital crimes have been moderated when it has seemed that the criminal could not reasonably be held responsible for his crimes, however dreadful. In Britain in the bad old days, juries in murder trials would frequently return verdicts of "guilty but insane" after careful instruction by the judge in the niceties of the *McNaughton* rules, intended to help decide whether a person carrying out a murder knew at the time the difference between "right and wrong". With the abolition of capital punishment, the need of this distinction has vanished, subsumed in two competing liberal principles — the view that people who commit what were once capital crimes are, by that test alone, out of their minds or mad, and the more subversive opinion (linked primarily with the name of R.D. Laing) that madness is simply a matter of the definition of normalcy, and thus a subjective matter. The Sutcliffe case has shown that British juries do not accept the first of these views and, fortunately, are probably ignorant of the second.

The result is an unhappy muddle, which cannot last much longer. The idea that the best way of treating serious psychiatric illness is to avoid, wherever possible, locking people up in hospitals has more than convenience and cost to commend it; institutionalization is, of itself, harmful even if on some occasions necessary. Helping people to lead more or less normal lives, however great the social cost and frequent the disappointment, does at least preserve the possibility of recovery from psychiatric illness. These are principles on which many societies are now agreed. Their practice is comparatively so recent, in the past quarter of a century, that a formal evaluation of the benefits would be premature. But it seems unlikely that the practice will be wholly abandoned of regarding the psychiatrically disabled as essentially no different from the rest of the population. One is, so to speak, presumed sane until proved otherwise.

Inconveniently, it follows that a diagnosis of psychiatric disability cannot logically be a synonym for diminished responsibility, whether in a sensational murder trial or in more ordinary circumstances. Psychiatric disability can of course be an extenuating circumstance, in a court of law or elsewhere, but that is a different matter. Both the prosecution and the defence at last month's unseemly trial at the Old Bailey would have been more



persuasive if they had grasped that truth. The rest of us have still to wake up to the consequences of what is, in effect, the abolition of the concept of madness.

At first sight, the most obvious implications are in the legal system. People such as Mr Sutcliffe must be given the benefit of the doubt even if they are thus exposed to more serious penalties. The courts also need more helpful criteria for telling when the nature of an accused person's behaviour is such that full responsibility cannot be assumed. For the rest of us, the most telling consequence of the erosion of the concept of madness is not that R. D. Laing's subversive doctrines deserve respect, but that psychiatric illness deserves to be recognized for what it is — common and pervasive.

Nuclear politics

The past two weeks may turn out to have been more than usually significant for the development of nuclear power in the Western world. The fact that recent happenings are, in their way, self-contradictory, should not obscure their importance.

In France, the new president, M. Francois Mitterand, persuaded his first Cabinet last week that the plan to build another nuclear power station in Brittany should be put off at least until he has had a chance to organize the referendum on the future of nuclear energy in France promised during his election campaign: cynics are already saying that the gesture may win some votes in the coming elections for the Assembly without restricting his freedom to do what he chooses later on.

In Hamburg, almost the opposite has happened. After a year of furious opposition to the Brinkdorf nuclear power station, the Mayor of Hamburg has chosen to resign rather than to continue to threaten the already fragile stability of the government of Chancellor Helmut Schmidt, to whose political party the mayor belongs. In the United States, the Administration (which is no longer new) may not have the time, the energy or the inclination to fight Congress for the funds needed to finish off Clinch River, the first full-scale breeder reactor in the New World. In Britain, the argument (by the Monopolies Commission) has been more rudimentary: should a nationalized utility monopoly be entitled to charge its customers more than they would otherwise have to pay because it believes that it should be building nuclear power stations for "strategic" if not for economic reasons? With all these uncertainties in the air, nuclear engineers would be looking for quite different kinds of jobs if OPEC, at its meeting last week, had not agreed that the price of oil should be increased by a little less than the inflation rate (in dollars) over the past six months.

This curious conjunction of disparate events is a telling reminder that the time has come when the relationship between governments and nuclear energy should be changed. For the past thirty-five years (since the ending of the Second World War), it has been taken as read that nuclear energy is too important to be left to nuclear engineers. There are some grounds for this position. Obviously governments, small as well as big, have an interest in the proliferation of nuclear weapons — they either wish to stop it or to make sure they are not left out. Governments also have an interest not easily distinguishable from a duty to ensure that their electors will not be put at hazard by the operations of nuclear plants. It is also proper that governments should have a view on the place of nuclear energy in the evolving pattern of energy consumption. But none of this justifies the detailed concern of most governments for the intricacies of their nuclear power programmes.

If nuclear power had been an ordinary technology, government would long since have withdrawn from detailed involvement. Originally they were dragged in to regulate the use made of fissile material and to finance the high cost of reactor development. Although safety has been an issue from the start, it is only in the past decade that governments have been compelled by political pressure to consider the general question that the nuclear industry may be inherently too dangerous to be accommodated even in an

advanced industrial society. President Mitterand's response is politically but not economically prudent — to halt the further development of nuclear power in France, and to hold a referendum; he knows that he will need the votes of as many French interests as possible to win a compliant Chamber of Deputies later in the year. The fact that it is the inverse of the 1979 response of Mrs Thatcher's then newly elected British government to general concern about nuclear energy suggests the unpalatable prospect that policies on nuclear energy are now politically partisan — right-wing governments build reactors, left-wing governments cry halt. That would be a recipe for doing nothing.

So there is an urgent need to rescue governments, as far as possible, from their political responsibility for nuclear power. How is this to be done? The prospects are not as hopeless as they sometimes seem. In the regulation of fissile material, the technical problems are well understood and the risks of failure keenly appreciated. Even so, on the principle that shedding administrative responsibility for nuclear matters is wise, governments should make fuller use the international safeguards that exist or could be created. The cost of nuclear development raises more serious difficulties. Reactors of the types now in commercial service are sufficiently well understood, technologically and otherwise, for decisions about their further exploitation to be left to electricity utilities and made on strictly commercial grounds. (This is not always simple, as shown by the recent criticism of the British Central Electricity Generating Board by the Monopolies Commission and by the Reagan administration's flat refusal last week to help with the cost of cleaning up at Three Mile Island.)

Utilities however are not in a position to support fast reactor development or experiments in thermonuclear fusion. It is proper that taxpayer's money should be used instead. It is essential that governments should distance themselves from the use eventually made of successful developments, insisting that success does not predicate widespread use. (Successive United States administrations have done this consistently, but the Clinch River reactor has also been hindered by doubts about cost and design).

But none of this will absolve governments of responsibility on the general question of safety. If a substantial part of an electorate is persuaded, even if mistakenly, that the civil nuclear enterprise is inherently unsafe, a democratic government has no choice but to grasp the issue firmly — if it wants to be re-elected. The question to be answered now in France is whether repeated declarations that nuclear energy is an abomination are to be taken at face value.

That there should be local opposition to the building of nuclear plants and that arguments about safety should arise is not surprising. The same happens when chemical manufacturers look for new sites. Would-be operators of nuclear plants must be prepared to allay reasonable doubts on safety. Then it is also right that planning authorities (whose authority derives in the last resort from central government) should arbitrate and if necessary override local opinion — otherwise, national interests must be perpetually subservient to local interests. Technically this is what will be happening next year, when the Central Electricity Generating Board will apply to build a pressurized water reactor plant in Suffolk. The snag is that the ultimate arbiters in the planning process will be members of the British government — the Ministers of the Environment and of Energy. Should they not also distance themselves from the process by saying in advance that they will accept whatever recommendations the judicial enquiry makes?

The wider question of whether there should be nuclear energy at all is much harder to resolve. President Mitterand's referendum is likely to create as much confusion as those in Sweden and Austria in the past two years. The overriding difficulty is that it is never possible to be certain that those declaring themselves against nuclear energy have been fully seized of the counter-arguments — the need for alternative sources of energy whatever they are, for example. On issues such as nuclear energy, decision by referendum is almost always bad decision — governments must shoulder the responsibility. What else are governments for?

NIH censure for Dr Martin Cline

Tighter rules for future research plans

Washington

Dr Martin J. Cline, the California scientist who has admitted using recombinant DNA molecules in unapproved experiments with human patients, has been rapped sharply over the knuckles by the US National Institutes of Health (NIH). The director of NIH, Dr Donald Fredrickson, announced last week that he has accepted the recommendation of an investigatory committee that Dr Cline be required to obtain special permission from NIH in future to carry out research using recombinant DNA techniques, the first time such action has been taken against an individual investigator.

Dr Fredrickson also agreed that review committees assessing future grant applications from Dr Cline be provided with a detailed account of his admitted transgressions in attempting to treat patients with thalassaemia. However, the investigatory committee, which described his actions as a clear violation of NIH guidelines covering recombinant DNA research, has not suggested stronger action.

Rather it has proposed — and Dr Fredrickson has accepted — that the directors and advisory councils of the three institutes from which Dr Cline at present receives grants totalling about \$600,000 a year should decide whether any of his current support should be withdrawn. Dr Cline's sentence therefore rests essentially in the hands of his scientific peers, a precedent-setting situation which is being closely watched by groups such as the President's Commission for the Study of Ethical Problems in Biomedical Research.

Dr Cline resigned in February from his position as chief of the division of haematology-oncology at the University of California, Los Angeles (UCLA), although he remains on the university faculty as professor of medical oncology. His resignation followed the admission that, in conducting experiments with patients suffering from β -thalassaemia in Italy and Israel last summer, he had transplanted bone marrow cells whose genetic material had been altered by recombinant DNA techniques. This was despite the fact that he had earlier told the hospital authorities that his proposed therapy would not involve such types of cell.

Dr Cline told the NIH committee that he was unaware that strict federal regulations requiring prior approval of experiments involving human subjects was required for any experiment carried out by university researchers, whether in the United States or abroad. The UCLA committee subse-

quently told NIH that the definitions of collaborating institutions contained in documents distributed to research staff "are not as clear as they might be".

It was the unauthorized use of recombinant DNA molecules, however, that made the charges against Dr Cline more serious. In written evidence to NIH investigators, he says he took samples of cloned genes intending to perform *in vitro* studies of β -thalassaemic marrow in Italy and, if possible, organize clinical trials in Israel and Italy.

On the last day of his visit to Israel, he received permission from authorities at the Mount Scopus Hospital of the Hassadah medical complex to use purified genes in experimental therapy with a 21-year-old girl suffering from β -thalassaemic major, a blood disease rare in the United States but more common in the Mediterranean region. Having originally intended — and received the permission — to carry out the experiments with unaltered genetic material, Dr Cline decided to subject bone marrow removed from the posterior iliac crest to a DNA-mediated gene transfer technique using recombinant genes which had been planned for the *in vitro* studies.

A similar procedure, in which the treated bone marrow was replaced in the patient in an attempt to stimulate the production of healthy red blood cells, was performed four days later on a 16-year-old girl with the same disorder at the University Polyclinic in Naples, Italy. Describing the Israeli experiment, Dr Cline later told NIH that he had decided to use the recombinant genes on medical grounds "because I believed that they would increase the possibility of

introducing β -globin genes that would be functionally effective, and would impose no additional risk to the patient, since it was known that pieces of DNA are efficiently linked in all combinations once they are taken into cells".

The medical authorities at the Israeli hospital, however, who had taken great pains to check that Dr Cline's proposed experiment did not involve the use of genetically altered cells, were upset at discovering what had taken place. Dr Cline's collaborator at Hadassah, Dr E.A. Rachmilewitz, denied any knowledge that recombinant DNA was being used and investigations carried out at UCLA confirmed that Dr Cline had acted on his own.

Accepting Dr Cline's letter of resignation, the dean of the UCLA School of Medicine, Dr Sherman Mellinkoff, accepted that no known harm had been done to the two patients, and that the experiments might prove helpful in treating others. But he added "It is also true that the freedom to conduct experiments of benefit to mankind is jeopardized by failure to act in accord with the relevant regulations in these circumstances".

Both Dr Cline and the university have declined to comment on the report of the NIH committee and Dr Fredrickson's subsequent decision. However, in a letter to NIH written in January, Dr Cline says "I greatly regret my decision to proceed with the use of recombinant DNA molecules without first obtaining permission from the appropriate committees", adding that "I exercised poor judgement in failing to halt the study and seek appropriate approval".

David Dickson

Social scientists take over ANZAAS forum

Brisbane

The 51st Congress of the Australian and New Zealand Association for the Advancement of Science (ANZAAS), held at the University of Queensland in Brisbane (11–15 May) was publicized as a "stocktake for science", but it turned out that the association itself was most under scrutiny. The New Zealand contingent has threatened to withdraw and go independent. The prominence of social science has increased confusion about the role of ANZAAS, which will be added to at the next congress by the introduction of new sections on law and robotics.

For basic and applied scientists, ANZAAS has become a forum for retrospective reviews and personal contacts but no longer an occasion for the announcement of original research results. The specialist professional societies of Australia have now completely taken over that job. Thus more than 600 members of the Australian Society for Microbiology attended its annual conference in Canberra in the previous week but only a few dozen

people turned up to the microbiology section at ANZAAS.

ANZAAS's own 10-times-a-year journal *Search* publishes only a minute handful of the 800-plus papers presented at each congress. There is little professional discipline over the speakers — papers are not vetted before delivery and too many speak from notes. Although many speakers took their responsibilities seriously, the overall standard was just too patchy. Presentation of a paper at ANZAAS may provoke valuable discussion, but it does not guarantee publication or significant enhancement of scientists' reputations.

For social scientists and some humanities, however, ANZAAS has become a welcome place for the communication of new research results and ideas. Given the science-and-society approach of some of the more traditionally scientific sections of ANZAAS the social sciences have now become dominant at this, Australia's largest annual academic meeting.

The organizers of the Brisbane congress

latched on to the popularity of energy as a topic for endless talk and took "Energy and equity" as the theme. Australia is going through an excitable phase over energy matters. Australia's huge coal reserves are proving immensely attractive to foreign interests. Oil shale deposits, notably in Queensland, were the subject of wildly optimistic predictions of national self-sufficiency in oil, until the dominant Exxon partners pulled back on grounds of massively increased cost estimates.

Unfortunately, the symposia which offered well-known people a chance to air their well-known views did not significantly advance the professional, popular or political understanding of the facts and issues. Full publication of these addresses is unlikely, and the brief mentions they received in newspapers caused little impact. The organizers failed to capitalize on the large media contingent present with the energy theme buried in a snowstorm of unrelated items. The paper which attracted most news reporting and comment — even a newspaper leader — was a sociological study of the sub-culture of nudist colonies.

ANZAAS congresses suffer from a lack of continuity. Each meeting is organized in a different city by a totally different group. ANZAAS also seems to have no collective memory, so that the failings of one meeting are repeated. Its central organization is weak because it depends too much on volunteers — its membership subscription income remains static and it receives no government subsidy.

The enrolment of 2,300 at the Brisbane congress was disappointing, being right on the financial break-even point. ANZAAS will therefore now have to struggle financially for another year in the hope that next year's meeting in Sydney will attract enough delegates to lift its spirits and wipe out its deficit.

For all its faults, however, ANZAAS remains a potent force in Australian (not New Zealand) academic life, which could realise its potential if only it could get its act together. This year's congress came only two weeks after Prime Minister Malcolm Fraser announced a series of cost-cutting measures recommended by a group of senior ministers, popularly known as the "Razor Gang". The Razor Gang slashed into the supposed "fat" of publicly funded science and did so to an extent probably greater than any other single area. An indiscriminating cut of 2–3 per cent in the staffing levels of all public service operations will hit CSIRO hard, though this has as yet not been publicly recognized. Another act of the Razor Gang — the imposition of fees for second degrees in universities — is the cause of nationwide protests by staff and students alike.

Fears for the future health of Australian science were all the talk at ANZAAS, but only privately. Not a murmur was heard publicly about these problems. ANZAAS 1981 may well be remembered as an opportunity lost.

Peter Pockley

Mitterand's new ministers On your Marx

With the all-important French legislative elections looming on 14 and 21 June — which will determine whether the new socialist president, François Mitterand, has real power or not — it may be unwise to read too much into the events of the past few days. But Mitterand's much-vaunted commitment to science seems in fact to be one to technology — albeit technology with a socialist mask.

Mitterand has appointed Jean-Pierre Chevènement, a 42-year-old left-wing intellectual and founder of the Centre for Socialist Studies, Research and Education (the broadly Marxist CERES), as Minister of State for Research and Technology. The other principal contender for the job, the scientist and director of the Institut Pasteur, Professor François Gros, has been appointed as scientific advisor to the Prime Minister, M. Pierre Mauroy.

Chevènement is a great admirer of the highly-centralized Japanese ministry for industry and technology, MITI; and as a Minister of State he will be a member of Mitterand's powerful inner cabinet of five. He will rank higher even than the Minister of Industry, M. Pierre Joxe (who, incidentally, also calls himself a Marxist).

Chevènement and Joxe are now locked in battle over who will control which of France's many scientific and technological institutions. The Délégation Générale pour la Recherche Scientifique et Technique (DGRST), which draws up guidelines and coordinates research throughout the government, but has only a small autonomous budget will be Chevènement's of right; and it seems he has also won control of the principal body funding basic

science in France, the Centre National de la Recherche Scientifique (CNRS), from the ministry of education. The Centre National pour les Etudes Spatiales (CNES) which controls France's scientific and technological work in space, may also be a major prize from the ministry of industry, and another could be the Agence Nationale de Valorisation de la Recherche (ANVAR), which promotes innovation in French industry. The control of the medical research body (INSERM) and the agricultural research council (INRA) is also in question.

Even the atomic energy authority (the CEA) might fall to Chevènement, but once the dust of Mitterand's energy policy has settled, the science and technology minister will be seen to be strongly pro-nuclear (see box). He is also greatly interested in nuclear weapons (he backs a French strategic nuclear capability), and in military research.

Where is socialism — let alone Marxism — in all this? It comes partly in nationalization: Mitterand has promised to nationalize nine big industrial companies, to give greater state control of major technical sectors of the French economy (this will be M. Joxe's preoccupation). It comes in promised efforts towards less centralized, more democratic control of technological decision making (Mitterand speaks of a referendum on nuclear power, for example). And it may come in Chevènement's professed concern for the impact of new technologies on employment and working conditions. His attitude to the problem of short-term contracts for young researchers and technicians, which has flared up regularly in the universities, CNRS, INSERM and INRA in recent years is not yet clear.

Robert Walgate

French face energy questions

Energy policy in France is entering a rather confused period. The new government has cancelled the bitterly contested plans for a nuclear power station at Plogoff in Brittany but has announced the start-up of two new 900 MW stations at Gravelines and Tricastin. And when the outgoing government in its dying days gave the go-ahead for doubling the size of the spent fuel reprocessing plant at Cap de la Hague there was no demur from the already-elected Mitterand.

So where lies the new French government's energy policy? There is no energy minister in the new government. The President, it seems, would like to keep this difficult card in French politics to himself. And so far, in fact, Mitterand's pre-election promises and his first actions as President have been consistent. He offered only a "pause" to reconsider nuclear power, in which reactors under construction (like Tricastin and Gravelines) would continue to completion; Plogoff has not begun. And Cap de la Hague has been so inefficient it needs

refurbishment to cope with future spent fuel.

But there is disagreement in his cabinet about what to do next. Some say Plogoff is cancelled; others, that it is merely postponed. Mitterand may call some kind of referendum (and if he does, is likely to find the French in favour of nuclear power) or he may opt for a parliamentary inquiry, through his promised committee on technology assessment, or he may hold a public inquiry.

But barring accidents like Three Mile Island, France's nuclear programme will probably survive the storms, because the country needs the energy, and soon. Despite the vociferous opposition at Plogoff last year, the electorate of the region in fact voted overwhelmingly for pro-nuclear candidates in the first round of the recent presidential elections. The (Giscardian) member of parliament for the region complained last week "where will it be possible at sure cost, to find 5,200 MW for Brittany by 1990? And another 4,200 jobs?".

Robert Walgate

Corporate largesse

More tax breaks?

A tax incentive measure designed to stimulate corporate support for scientific research in US universities has taken a first step towards congressional approval. A comprehensive tax bill sponsored by Representative James M. Shannon has passed a House Ways and Means Committee panel on its way to the full committee.

Representatives from major universities, including the presidents of the Massachusetts Institute of Technology and Stanford, have testified at House hearings along with prominent industry members in support of the legislation. They hope that the Shannon bill will offset the expected losses from the Reagan budget by attracting more than \$500 million to university research next year.

Three sections of the tax incentive plan would specifically benefit universities:

- A 25 per cent tax credit for contributions from corporations to a "research reserve fund" that would guarantee against shortfalls in research funding. This money, which could be up to 5 per cent of a corporation's income, can remain in the fund for four years drawing interest, while the taxpayer receives more credit for additional money added.

- A 25 per cent tax credit for money contributed to any form of research and development at universities or private laboratories if the total contribution exceeds the average amount given by that corporation over the previous three years.

- Tax deductions to corporations giving charitable contributions of scientific or educational equipment, based on the market value rather than production cost.

The "research reserve fund" would actually give businesses a larger tax credit because a contributing corporation would receive an additional deduction of up to 46 per cent when its money was transferred from the fund to a specific university.

Opposition to the bill comes from those who believe it would entail a major loss of federal revenue (around \$207 million), but the White House has indicated in informal discussion with House members that it would definitely consider supporting it.

Besides bringing more corporate dollars to American academic research, which at present makes up less than 5 per cent of the total given to universities, supporters of the bill say it would foster closer ties between university scientists and applied research.

Passage of the bill may, however, be hindered by the lack of a unified academic voice. Some smaller universities feel that it will hurt them because most money will be directed towards the larger institutions. But insiders say the bill has a bright future because Senate Finance Committee chairman, Senator Robert Dole, has expressed great interest in its passage.

Michael D. Stein

Yugoslavian university unrest

Cuts feared

The University of Pristina, the third largest university in Yugoslavia, may be cut to about one fifth its present size following the political unrest of the last three months, which began in the university.

Pristina is the capital of the autonomous province of Kosovo which forms part of the Republic of Serbia but enjoys considerable local self-government. It ranks as one of the less developed areas of Yugoslavia, and hence, over the last few years, has benefited considerably from federal development schemes. Under such a scheme, the University of Pristina has grown during the last decade to a student body of almost 50,000, instead of the 10,000 originally planned, an expansion deemed necessary by Belgrade for "the overall development of Kosovo and the advancement of national equality". (The population of Kosovo is predominantly Albanian).

However, the growth in numbers was not matched by a commensurate expansion of student facilities, and on 11 March there was a small demonstration of students demanding improvements in hostel accommodation and canteen food. The local and university authorities seem to have over-reacted when restoring order and almost inevitably, the demonstrations escalated and became more political in nature. By the end of April, demonstrators were demanding outright secession of Kosovo from the federation and even a link-up with Albania; cultural and scientific exchange programmes between Yugoslavia and Albania were subsequently cut.

Although by now the demonstrations had spread far beyond the student body, the university was still regarded as their focus. Accordingly, on 18 May, Pazajit Nusi, vice president of Kosovo Assembly's executive council, and Dr Imer Jaka, Secretary for Education, Science and Culture, resigned. The same evening there was another on-campus demonstration, and the next day the executive council ordered the university and all other institutions of higher education in Kosovo to close for the vacation.

The university party organization, in the meantime, had set up its own action committee. According to one report more than 500 members of the philosophical faculty have been expelled from the Party, and four persons suspected of organizing the demonstrations have been expelled from the university. Also, various abuses in the university structure have been uncovered. At the same time, the action committee was highly critical of the Belgrade authorities and media for exaggerating the situation, and was particularly critical of Milan Daljevic, Executive Secretary of the (Federal) Central Committee Praesidium, who had

spoken of the University of Pristina as "a fortress of nationalism" and had alleged that not a single professor had made any political statement condemning "enemy actions". An official protest has been sent to the Central Committee Praesidium.

To offset the incomplete, and often incorrect, media coverage, the action committee called for a complete dossier on the unrest to be prepared.

With so few jobs available for existing graduates and 55,000 young people now receiving higher education in Kosovo, the suggestions from Belgrade that the university be cut back to its original 10,000 places and funding switched to other educational needs, poses a real threat to the University of Pristina. So far the action committee has made no explicit reference to the suggestion, but has merely suggested that future admissions be tailored more closely to "social needs".

Vera Rich

US security worries

No compromise

Washington

In a move reflecting deep-seated disagreement over how far national security arguments should influence the procedures of basic research, scientists on two advisory committees to the National Science Foundation (NSF) have criticized proposals by the American Council on Education (ACE) that papers containing potentially sensitive results in computer research be voluntarily submitted to national security authorities for review before publication.

The proposal was made by a study group on public cryptography set up by ACE at the suggestion of the National Security Agency (NSA). This followed a series of incidents in which NSA had sought to restrain the dissemination of unclassified data on the grounds that they could threaten national security by assisting the development of techniques to make and break codes.

NSA already accepts in principle the proposals of the study group, and has outlined to several professional organizations and their scientific journals the group's conclusions under which suggestions for changes, deletions or publication delays would be made on manuscripts submitted to the agency. ACE announced last month that "the intelligence and academic communities have agreed on guidelines for voluntary review before publication of computer cryptography articles". However, members of the mathematical and computer sciences advisory committee of NSF have said that voluntary submission to pre-publication review represents a "direct and serious threat to NSF's charter of furthering basic scientific research". A report, prepared by a subcommittee under the chairmanship of Dr John Guttag, says that the conclusions of the ACE study

group "reflect a view of 'national interest' and 'national security' that is derived largely from concerns related to the government's need for military and diplomatic security". Other factors which it says should be considered include the competitive needs of the US information processing industry, and the need to protect individuals from unwarranted invasions of privacy.

The two advisory committees decided last autumn to investigate the role of NSF in supporting cryptological research, following an incident in which a computer research scientist who, having applied to NSF for a grant, was told that NSA was interested in supporting his research — even though he had not approached the security agency. In general, the committees endorse the policy outlined at the time by NSF deputy director Dr Donald Langenberg.

However, the committee's report takes issue with Dr Langenberg's remark that imposing more stringent reporting requirements on scientists should be viewed "simply as a change in administrative practice". Rather, it says, any change in current practice should be considered a significant shift in policy which could represent "serious interference with the conduct of research".

The committees also object to the letter being sent to those who are awarded cryptography grants notifying them that they should seek prior review by NSF of any results which might have national security implications. The report believes this would adversely affect research and proposes instead that a description of the research results should be forwarded to NSF or to any other government agency designated by the foundation — at the same time as its dissemination to other scientists or publication.

The NSF report points out that NSA has proposed spending between two and three million dollars a year on unclassified research — "We doubt that this much money could be spent productively supporting academic work on cryptography and cryptanalysis, and imagine that NSA will end up funding and taking an active interest in a relatively wide variety of research projects". And it warns of the potential dangers of NSA becoming the principal source of funding for cryptology research as the Defense Advanced Research Projects Agency has done in fields such as artificial intelligence and systems research.

The Association of Computing Machinery (ACM) is also concerned over moves in Congress to give the federal government greater powers to control the publication of scientific or technical data. In particular, ACM has criticized a bill introduced by Representative Charles E. Bennett of Florida which would extend existing controls on "militarily critical" technologies to cover not only hardware, but any related technical information.

Dr Peter Denning, the president of ACM, feels that the bill would extend to many areas of research the type of "born classified" restrictions now applied to nuclear research under the terms of the Atomic Energy Act, and used as the basis for the (unsuccessful) prosecution last year of the *Progressive* magazine for publishing details of how a hydrogen bomb works.

The Reagan administration's attitude to this whole area is uncertain. Mr Lawrence Brady, nominated to head the Commerce Department's office responsible for export controls, promised a Senate committee last month that a new East-West trade policy, expected from the White House in a few weeks, would include rigorous new criteria for screening critical technology.

Mr Brady's appointment, however, has worried representatives of several multinational companies who feel that his zeal in restricting high technology exports to the Soviet Union may prove costly to them. The Administration is trying to define an acceptable compromise between the high priority it has given "national security" arguments, and the freedom being demanded by both technology exporters and the academic community. The sharp division between the ACE study and the NSF advisory committee indicates that such a compromise may still be far away.

David Dickson

UK agricultural research

Change at the top

The recent appointment of two chief scientists to the British Ministry of Agriculture, Fisheries and Food is expected to increase the ministry's influence on research supported by the Agricultural Research Council. But there is to be no formal change in the relationship; this has been ruled out by the Secretary of State for Agriculture, together with the possibility that more money should be transferred from the council to the ministry for spending on commissioned research.

These developments help to clarify more than a year's uncertainty about the future relationship between the two organizations. After the House of Commons Public Accounts Committee recommended in July 1979 that the ministry should have greater control over the council's research, a committee under the chairmanship of Sir Brian Hayes, permanent secretary at the ministry, was set up to decide what should be done.

One of its conclusions was that the Rothschild principle, whereby almost half of the council's research is commissioned by the ministry, is to remain intact. Another was that the ministry could exert more influence if it coordinated its own science policy more effectively. To that end, the former chief scientist, Professor B. G. Weitz, has been replaced by two chief scientists, Professor Frank Raymond for agriculture and horticulture and Dr George

French to make Arabsat

The French aerospace firm la Société Nationale Industrielle Aérospatiale (SNIAS) has won the coveted contract for "Arabsat" — a clutch of three communications satellites with an option on a fourth, for the Middle East — in competition with British Aerospace and the Hughes Corporation. But hidden behind SNIAS is Ford Aerospace, which will get 55 per cent of the \$134.3 million deal. Until recently Ford was under an Arab boycott, and the company clearly thought it politic to work with a French partner, French-Arab friendship having been so strong under President Giscard d'Estaing.

British Aerospace are understandably downhearted. The firm has so far sold satellite systems only to the European Space Agency (ESA), and it saw Arabsat as a potential breakthrough into new markets.

But British Aerospace still have orders for seven satellites worth £100 million, which will keep them busy until 1985. The company is building five communications satellites for ESA, each capable of carrying 12,500 telephone conversations and two television channels. These are bigger than the Arabsats which will be designed for 8,000 telephone lines. British Aerospace are also building two maritime "Marecs" satellites for ESA, the first due to be launched by Ariane in October.

Meanwhile SNIAS is crowing just as British Aerospace would have done if it had won Arabsat. It is the first time a French aerospace firm has won the place of prime contractor for a foreign satellite system. SNIAS now have their eye on Australia, Columbia, Mexico and Brazil.

Robert Walgate

Elton for fisheries and food.

The idea is that two chief scientists will enable the ministry to make better use of the Agricultural Development and Advisory Service (ADAS), which accounts for almost half of the ministry's staff and advises farmers and officials on technical aspects of agricultural and horticultural practice. Professor Raymond will report to ADAS and formulate an appropriate policy for research. Thus the influence of ADAS within the ministry will also be enhanced. Dr Elton will concentrate on fisheries and food research. Both chief scientists will sit on the Agricultural Research Council.

Precisely how the new arrangements will affect the council remains to be seen. But the outcome contrasts sharply with the situation for medical research: last October, the Medical Research Council won back most of the money transferred to the Department of Health, so doing away with the Rothschild principle.

Judy Redfearn

Origin of Species

Premises, premises

Origin of Species is the latest in the series of new permanent exhibitions scheduled by the British Museum (Natural History). Under any circumstances, it would be an important exhibition, lying at the heart of what the museum is trying to explain. Unless there is some central thread of theory linking together louse and lousewort, *Archaeopteryx* and albatross, bushbaby and bushman, the exhibits that surround us in the museum are of no greater significance than a collection of postage stamps — interesting for their variety, occasionally beautiful, but intellectually sterile. But circumstances today make us look even more closely at such an exhibition, for evolutionary theory seems to be assailed ever more fiercely by its enemies, and to be only half-heartedly defended by its supporters.

Let's start, though, with the presentation and packaging, which is what will (or will not) attract the visitor in the first place. The Origin of Species exhibition is mounted in the western wing of the premises designed by Waterhouse. Spacious, elegant and well-windowed, it is a beautiful foil for the exhibition, which is arranged in eleven sections, each presenting a different aspect of the theory of evolution by natural selection: the species, competition, variation, inheritance and so on. With the inevitable few minor reservations, I thought that this system worked very well: each concept is comprehensible and is clearly and attractively explained. Both diagrammatic and real-life presentations are used, and are cleverly intermingled. There is also a natural selection computer game, designed to appeal to the Space Invaders generation. Finally, there are four mini-theatres in which film-loops explain different aspects of the theory.

Understanding the details of Darwin's theory is, however, only one part of what we scientists hope our museums will do for us. In the face of the organized pressures of religious and mystical sects, evolutionists need some organization to represent *their* views, no less fervently held, as cogently as possible. Not that it should descend to the half-truths and double-talk of political propaganda. But it should suit the terms of its message to those who will listen to it, rather than blunting its edge with the hair-splitting logic-chopping of the philosophy of science. Here, I fear, the BM (NH) has failed us.

"The Survival of the Fittest is an empty phrase; it is a play on words. For this reason, many critics feel that not only is the idea of evolution unscientific, but the idea of natural selection also. There's no point in asking whether or not we should believe in the idea of natural selection, because it is the inevitable logical consequence of a set of premises." "The idea of evolution by

natural selection is a matter of logic, not science, and it follows that the concept of evolution by natural selection is not, strictly speaking, scientific." "If we accept that evolution *has* taken place, though obviously we must keep an open mind on it" "We can't prove that the idea is true, only that it has not yet been proved false." "It may one day be replaced by a better theory, but until then" These are all quotations from the film loop in which the present status of the theory of evolution is explained to a layman by a scientist. If this is the voice of our friends and supporters, then Creation protect us from our enemies.

Of course, to a scientist, evolution and natural selection are "only" theories — but how much real doubt is implied by that statement? The philosopher of science may utter his customary caution "Anything you say will be taken down; it will only be falsifiable, and can never be proved true". But these theories have withstood over a century of testing, by a great variety of methods unimaginable to Darwin. These methods not only all support a theory of evolution in general fashion, they combine to support a single, precise version of the relationships of living things. For example, palaeontology, biogeography, serology, protein sequencing etcetera, *all* suggest that man is most closely related to the African apes, rather than each type of evidence pointing to a different branch of the tree of life. The significance of Mendel's work was not merely that it did *not* support Lamarck (as the film-loop tells us), but also that it was exactly what Darwin's theory demanded and had, to that extent, effectively predicted (which the film-loop does *not* tell us). How many biologists really think that, one day, they will open *Nature* and find a new, completely different theory of evolution?

Equally, if we say that Darwin's theory may one day be replaced by a "better theory", do we really mean anything *completely* different? In fact, of course, few biologists consider it remotely possible that the great variety of evidence that supports this theory could suddenly be rearranged to provide an even more persuasive support for a creationist theory. But that is not all. We don't even think that it could support a dramatically different *scientific* theory, in the way that earlier observations of the heavens were transformed from being compatible with an Earth-centred Universe to demonstrating a Sun-centred Solar System. Genetic drift or neo-Lamarckism may lead to changes of emphasis or provide explanations of the exceptional or the rare, but they show no signs whatever of taking over the central ground of our science. Though no biologist can deny the possibility that God created man, few

Huxley on *Nature*

At the celebrations of the centenary of the move of the British Museum (Natural History) to the building it now occupies in South Kensington (London), the president of the Royal Society, Sir Andrew Huxley, devoted the major part of his speech to an attack on *Nature* for giving publicity to criticism of the museum (of which Sir Andrew is now a trustee) in recent months.

Sir Andrew complained that *Nature* has "printed more than 30 letters centering around attacks on the scientific thinking of members of staff of the museum" and said that the correspondence, especially that relating to cladistics, would have been "unintelligible to 99 per cent of the readership of *Nature*".

The "obscurities and irrelevances" of the debate were "in cloud-cuckoo land". Sir Andrew said that *Nature* published such letters for the same reason that *The Times* of London devoted "so much space to the Sutcliffe trial" (a recent sensational murder trial in Britain) — "their readers enjoy these things".

Sir Andrew also accuses *Nature* of having published a leading article about the museum "on the basis of a few words" taken out of context, and of not having published "a brief reply from the museum". Sir Andrew warned *Nature* that "crying wolf will drive its readers into disregarding its future warnings" and those "wounded by these articles" should ignore them.

would doubt that, if He did so, the mechanism that Darwin discerned was the one that He chose to use. It is the strength of conviction of working biologists that needs to be accurately translated to the man in the museum gallery, not the technical caveats with which we remind ourselves of the potential weaknesses of more recent or less wide-ranging theories. And let us not forget that Dr Roger Miles, head of the museum's Public Services Department, is quoted as saying that their exhibitions are aimed at the average 15-year-old, not at scholars.

Lest I be accused of losing my sense of proportion, let me repeat that I found most of the exhibition appealing, satisfying and clear. If I take issue at length with one small part of it, it is because I consider that part to be crucial. This film-loop will be studied primarily by those few people who are really interested in trying to find out precisely how the world of science views this most important and wide-ranging explanation of the phenomena of the living world, and they may judge this against the less dispassionate appeals of such groups as the creationists. Just as war may be too serious to be left to the military, so the presentation of science is too crucial to the scientist to be left wholly to the mercy of the populist presenter.

Barry Cox

CORRESPONDENCE

Apes or angels?

SIR — The description of the punctuated equilibrium model as Marxist¹, although correct, obscures its real importance in the thinking of east coast radicals: the theory is at root anti-racist. As the debate between Rose² and Dawkins³ made clear, the radical scientific opposition to racism requires a denial that there is any genetic variation of any significance from place to place within the human species. As there is a considerable amount of geographical variation in anthropometric characters and in the frequencies of blood groups and other biochemical traits, anyone who wishes to espouse both the neo-Darwinian synthesis and this form of scientific anti-racism must adopt the argument either that the genes affecting behaviour just happen not to be subject to any effective geographical variation in their distribution⁴, which is uncomfortably close to special pleading, or that behaviour is not under genetic control. As this second proposition is known to be untrue for other animals, there is an urgent need for a general theory that will rescue scientific anti-racism from sophistry.

The punctuated equilibrium theory^{5,6} holds that all species make qualitative "leaps" at the time they originate. This can be applied to humans as meaning that the particular and special "leap" made by our species was largely in freeing the functioning of the brain from genetic influences: no matter how gene-distributions may vary geographically, or hormones between the sexes, the psyche is entirely buffered against these and is influenced only by the environment⁷. Further, human races are nowhere near to speciation and hence, not having "leapt", are fundamentally "the same". The punctuated equilibrium theory is thus the modern radical version of Disraeli's famous question and answer "Is man an ape or an angel?". Gould is on the side of the (evolutionary) angels.

However, a belief in the absolute moral value of one's own theological (or scientific) position carries its own danger of personal damnation; this is the lesson of all witch-hunts. Although Gould⁸ would like us to believe that he is the potential victim this, in view of recent history, is a piece of *chutzpah*. Within the academic community it is those scientists whose theories could be conveniently labelled "racist" or "right-wing" who have been subjected to unofficial but nonetheless unpleasant persecution (the assault on Eysenck).

Most scientists do not, as Gould⁸ implies, resent the implication that their theories may be founded in their socio-political world-view: they simply regard this obvious fact as irrelevant. The argument that a theory is incorrect merely because the proponent has ulterior motives for holding it has been known as a species of false logic since the ancient Greeks. However, as radical scientists just do not accept the fact-value separation which Dawkins³ and other liberals use to defend their position, but rather adopt the Marxist view that moral statements are founded in objective reality (the only true science is that which serves the good of the people)⁹, they interpret the moral statement that racism is

wrong as implying the scientific statement that sociobiology is wrong. And as the Soviet ambassadors in Islamic countries have been trying to explain recently, it is important to distinguish a progressive force which swims with the moral tide of history from a reactionary imperialist force which swims against it. Perhaps the same distinction applies to witch-hunts.

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1. Halstead, L.B. *Nature* **288**, 208 (1980).
2. Rose, S. *Nature* **289**, 335 (1981); **290**, 431–432 (1981).
3. Dawkins, R. *Nature* **289**, 528 (1981).
4. Cavalli-Sforza, L.L. & Bodmer, W.F. *The Genetics of Human Populations* (W.H. Freeman, San Francisco, 1971).
5. Stanley, S.M. *Macroevolution, Pattern and Process* (W.H. Freeman, San Francisco, 1979).
6. Lewin, R. *Science* **210**, 883–887 (1980).
7. Futuyma, D.J. *Evolutionary Biology* (Sunderland, Massachusetts, 1979).
8. Gould, S.J. *Nature* **289**, 742 (1981).
9. Brecht, B. *Leben des Galilei* (Frankfurt, 1955).

It wasn't me

SIR — You recently published a letter on genetic determinism from someone in Massachusetts with a name remarkably similar to mine. My acquaintances all seem to think that I wrote that letter, so I wish to state publicly that this is not the case. His first name is spelled "Isadore".

ISIDORE NABI

University of Chicago,
Illinois, USA

Unsöld on Einstein

SIR — I feel that the article "German physics in row about Einstein" (*Nature* 16 April, p.535), dealing with a lecture and a publication of mine, needs some correction. It states that I began my "attack on Einstein" at a symposium held last May. In fact, this refers to a lecture, "Evolution of cosmic, biological and metal structures", which I gave on 25 April 1980 at a physics colloquium at the University of Marburg. With regard to the evolution of mankind, I mentioned the alarming problem of weapons and actions of mass destruction (gas warfare, the atomic bomb, napalm, atomic and neutron rockets, and, of course, Hitler's genocide), for which highly intelligent people were responsible. In the discussion which followed, no one, from an audience of some 200, said anything about Einstein. However, weeks later, a Marburg professor wrote to many physicists, complaining bitterly about my lecture.

As to my article in *Physikalische Blätter* dealing primarily with the Einstein celebrations of 1979, I, of course, fully acknowledge the great discoveries of Einstein, but I strongly disagree with the many statements which attempt to show him as a great politician or even a kind of saint. And I disagree still more with a prominent speaker who claimed that, compared with Einstein, Planck's political attitude was "childlike".

Being no historian, I took almost all my biographical information from the book *Einstein, the Life and Times* by Ronald W. Clark, a prominent British historian of

science. And the president of the Society for History of Sciences, Professor F. Krafft, stated in a letter of 29 April 1981 that, as far as he could see, my article contained no false statements or quotations.

The publication of my article was solely the responsibility of the five editors, one of whom was Professor Rollnik. Any attempt to shift the editorial responsibility to others would be quite unfair.

As to the reaction of others, I would like to emphasize that I received many letters of support from prominent scientists, while it seems Professor Rollnik received most of those which disagreed with me. Several of them seem to use the term "Nazi" in exactly the same way the Nazis used the term "White Jews", in order to outlaw all theoretical physicists in Germany!

ALBRECHT UNSÖLD

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University of Kiel, FRG

SIR — Under the West German press laws I am the editor responsible for the *Physikalische Blätter*, the journal of the German Physical Society, and would like, in this capacity, to say a few clarifying words on the article "German physics in row about Einstein" (*Nature* **290**, 535; 1981).

I intentionally did not involve Professor Rollnik, the president of the German Physical Society and co-publisher of the journal, in the discussions on the publication of Professor Albrecht Unsöld's manuscript as I do not consider it a good thing for the president of a scientific society to decide on the views, political or otherwise, of the society's members. A society's journal, which reflects only the opinions of the president, would indeed be a questionable enterprise.

I therefore much regret that Herr Rollnik has been rebuked. Why did the critics not write to Professor Unsöld or to the editorial staff?

Unsöld has received many letters agreeing with him and this endorsement largely accords with my reasons for deciding to publish the article: in no way did Unsöld want to enrich the Einstein biographies. He simply wanted to take Einstein (and Haber) as examples to illustrate that the trend towards increasingly more effective means of mass destruction, however well-intentioned they are subjectively, ultimately only enlarges the catastrophe. Unsöld destroyed the *idol* "Einstein" in order to get at the main theme of his article, the increasingly dangerous disproportion between man's moral capacities and his intellectual faculties. I am probably not the only one to share this concern with him.

Unsöld speaks expressly of the great "tragedy" of nationalism and how this also involved great natural scientists. This was not meant to discredit, but nor was it intended as antisemitism. Surely it was also tragic that nuclear weapons were developed by leading scientists of a people Hitler had horribly plagued. Nevertheless, this is of little help to us in Central Europe if these weapons are to be used in our countries. Not even the 30,000 Jews who still, or once again, live in Germany could hope to be spared.

KARL KROMPHARDT

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NEWS AND VIEWS

Geophysical controversy in the Gulf of Aden

from R.B. Whitmarsh

THE concept of seafloor spreading has been used successfully to unravel the history of the major ocean basins in terms of varying speeds and directions of spreading of lithospheric plates including, in some cases, periods of slow or negligible spreading. There remains, however, the problem of understanding the processes whereby a continent is split apart to produce a new ocean. For this reason there has been interest for some years in studying nascent or very young oceans, of which the best examples are probably the Gulf of Aden and the Red Sea. These represent the westward extension of the Carlsberg Ridge from the north-west Indian Ocean, and pass northwards into the sinistral Gulf of Aqaba/Dead Sea shear or transform fault.

The oceanic nature of the Gulf of Aden crust was established in 1966 by Laughton¹ on the basis of seismic refraction data. The presence of a mid-ocean ridge running down the centre of the Gulf was convincingly demonstrated by the presence of a central rough zone with large magnetic anomalies, offset by NE-SW fault zones, and the existence of a chain of earthquake epicentres and high heat-flow observations. Laughton did not attempt to date the oceanic crust which, on the basis of east-west faults exposed ashore, appeared to be younger than late Eocene to early Miocene. However, the existence of a central topographical rough zone, flanked by crust covered by 0.5–1.5 km of sediment, caused him to suggest two phases of opening of the Gulf.

Normally geophysicists date the oceanic crust by subjectively matching a sequence of seafloor spreading magnetic anomalies with a section of the geomagnetic reversal time scale which complies with the constraints offered by independent geophysical and geological information, such as palaeontological dates based on core samples. The difficulties and limitations of this method, when the anomaly sequence is short, have recently been amply demonstrated by the publication of two different spreading histories for the Gulf of Aden^{2,3}.

Cochran³, after Laughton *et al.*⁴ but with more data, favours a single phase of NE-SW seafloor spreading for the past 10 Myr. In both these papers the data base consisted of a set of variously oriented profiles from all parts of the Gulf. On all profiles, but particularly in the western and central Gulf, the anomaly amplitude decreases markedly away from the central anomaly. Fairly convincing correlations of calculated (synthetic) and observed anomalies can be obtained out to anomaly 5 (10 Myr BP) east of 45°E. West of this longitude the anomalies only fit well out to 3 or 4 Myr BP. Cochran's synthetic profiles do not convincingly reproduce the amplitude variations which occur away from the spreading axis.

Girdler and colleagues^{2,5} favour a more complex history of the Gulf of Aden involving at least two (and possibly three²) phases of seafloor spreading. Their data set consists of 16 magnetic profiles, obtained in a single survey and carefully oriented along the spreading direction, which cross the spreading axis between 43° and 46°E in the western Gulf of Aden. Like Cochran they believe that spreading has been continuous for about the past 5 Myr and they too could not identify anomalies older than anomaly 3 generated by the spreading axis west of 44°20'E. The difference in their interpretation lies in the proposal that there was an earlier phase beginning 23.5 Myr ago, followed by an 11.5 Myr hiatus in spreading from 16 to 4.5 Myr BP. Also, using geophysically plausible arguments about the decrease of magnetization with age in the upper crust, they were able to model successfully the amplitude variations in their survey area. Girdler and co-workers have attempted to choose a spreading history which is consistent with other geological and tectonic information; in particular, their two-phase model fits the last two major phases of transform motion along the Dead Sea rift as identified by Freund.

The disagreement between Cochran and Girdler's group lies simply in the interpretation of a 50 km-wide strip of magnetic anomalies, each side of the central spreading zone, which is at least 5 Myr old. As Cochran shows, profiles calculated from the two models are

qualitatively very similar out to his oldest identified anomaly. This surely indicates the futility of trying to resolve the problem by modelling seafloor spreading anomalies. Outside the above strip Girdler's group were able to model other anomalies for a further 50 km but these were of low amplitude and were evident on only some of their profiles.

As the oceanic lithosphere spreads away from a mid-ocean ridge, it cools and contracts and its upper surface (the acoustic basement on reflection profiles) follows a characteristic age versus depth curve⁶. Therefore it is implicit in the Girdler 11.5 Myr hiatus that a step should appear in the acoustic basement of about 450 m. Unfortunately, the roughness of the acoustic basement in the Gulf of Aden is such that an offset of this size in the mean level of the basement is barely resolvable and difficult to demonstrate convincingly. On the other hand, Cochran quotes depths of the basement producing his oldest anomaly which are apparently consistent with a continuous spreading history for the past 10 Myr.

As well as regional geological information concerning the ages of tectonic events on adjacent plate boundaries, there is the evidence of a Glomar Challenger borehole at site 231, some 150 km from the spreading axis. Here in the southern part of the central Gulf of Aden fossiliferous chalk about 13 Myr old was found embedded within the basaltic basement⁷. This age, while consistent with Cochran's interpretation, conflicts with an estimated basement age of 25 Myr BP from

1. Laughton, A.S. *Phil. Trans. R. Soc. A* **259**, 150 (1966).
2. Girdler, R.W., Brown, C., Noy, D.J.M. & Styles, P. *Phil. Trans. R. Soc. A* **298**, 1 (1980).
3. Cochran, J. *J. geophys. Res.* **86**, 263 (1981).
4. Laughton, A.S., Whitmarsh, R.B. & Jones, M.T. *Phil. Trans. R. Soc. A* **267**, 227 (1970).
5. Girdler, R.W. & Styles, P. *Nature* **271**, 615 (1978).
6. Sclater, J.G., Anderson, R.N. & Bell, M.L. *J. geophys. Res.* **76**, 7888 (1971).
7. Fisher, R.L. & Bumce, E.T. *Init. Rep. DSDP* **24**, 17 (1974).
8. Roeser, H. *Geol. Jahrb.* **13**, 131 (1975).
9. Searle, R.C. & Ross, D.A. *Geophys. J. R. astr. Soc.* **43**, 555 (1975).
10. Vine, F.J. *Science* **154**, 1405 (1966).
11. Noy, D.J. in *Tectonics and Geophysics of Continental Rifts* (eds Ramberg, I.R. & Neumann, E.R.) 279 (Reidel, Holland, 1978).

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the Girdler model. It is conceivable, however, that the basalt represents an intrusive sill, thereby post-dating the underlying crust.

Because horizontal, and particularly strike-slip, motion across the East African Rift is believed to have been negligible in the past few tens of millions of years, a corollary of any model of opening for the Gulf of Aden is that essentially the same history must fit observations in the Red

Sea. In the latter, the magnetic field also displays high-amplitude axial anomalies which can be correlated out to about 3–5 Myr BP^{8–10}. Here too, Girdler's group favours a two-phase spreading history¹¹ but this has yet to be matched precisely with their latest history for the Gulf of Aden².

The published evidence does not favour one model more than the other. The difficulties of interpreting short magnetic profiles are evident, although a systematic

magnetic survey recently conducted in the eastern Gulf of Aden where the spreading rate, and therefore the resolution of magnetic profiles, is greatest could yet provide conclusive evidence. The problem provides a salutary lesson on the necessity of combining the best geophysical and geological data and the difficulties which can arise if evidence from a single observational technique is used to study the Earth. □

The transmission and control of gonorrhea

from Robert M. May

IN the United States, roughly one million cases of gonorrhea are reported each year, and the Center for Disease Control suggests that between two and three million new cases may actually arise annually. An estimated 10–17 per cent of women with gonorrhea develop pelvic inflammatory disease (PID), which is the most common complication of the disease and is the leading cause of sterility in women. It is claimed¹ that direct and indirect costs related to PID and associated ectopic pregnancies exceeded 1.25 billion dollars in the United States in 1979.

For diseases such as measles, chicken pox or influenza, people recover relatively rapidly and thereafter become immune. Thus the average fraction of the population that is infected (the prevalence) cannot increase indefinitely, but rather saturates at a level where most contacts made by an infected person are with individuals who have acquired immunity. For these diseases, the prevalence therefore depends on the rate at which new susceptibles appear in the population (by birth or immigration), and it tends to be necessary to have large aggregations of people — typically 500,000 or more — in order to maintain the infection.

In common with most other sexually transmitted diseases, gonorrhea has characteristics causing its epidemiology to be quite different from the above infections. First, only sexually active individuals who could be infected by their contacts need to be considered as candidates in the transmission process. In contrast with simple models for infections such as measles or the common cold, a doubling of the population density does not double the overall rate at which new infections are produced by infected individuals (unless one makes the bizarre assumption that doubling the population density doubles an individual's typical rate of acquisition of sexual partners). Second, many infected people, especially women, are essentially asymptomatic and therefore do not seek treatment. Since individuals do not recover spontaneously from gonorrhea until a relatively long time has elapsed, such infected persons will remain infectious

until they do receive antibiotic treatment. Third, little or no immunity appears to be acquired on recovery from gonorrhea, so that most treated individuals rejoin the susceptible class as soon as the effects of antibiotics wear off. All this adds up to an infection that is admirably well adapted to persisting in small, low-density aggregations of humans.

The first mathematical model to cater specifically to these features of the epidemiology of gonorrhea was developed by Cooke and Yorke². Subsequent studies by Reynolds and Chan³, Hethcote⁴, Constable⁵ and others have attempted to estimate the parameters in models involving sets of coupled differential equations. Much of this work is reviewed by Yorke, Hethcote and Nold⁶ (hereafter YHN), who offer many important insights; their presentation is aimed at public health workers and is deliberately uncluttered with mathematical details.

YHN emphasize that, for gonorrhea, saturation effects arise when infectious individuals contact individuals who are already infected from some other source. They call this 'preemptive' saturation, and suggest that "preemption appears to be the only saturation factor that can affect a disease such as gonorrhea for which infection does not confer immunity".

Turning to the data for gonorrhea in the United States, YHN estimate the annual incidence to be 2.6 million cases per year, and the average duration of infection for women and men to be 55 days. This corresponds to a prevalence of around 400,000 cases at any given moment. Further assuming the 'at-risk' population of sexually active people to be around 20 million, YHN arrive at the result that approximately 2 per cent of the at-risk population is infected at any one time; changing the estimate of the at-risk population to 10 or to 50 million does not affect the essentials of the subsequent argument.

Given that only around 2 per cent or so of the contacts of an average infective

person are themselves already infected, preemption cannot be a significant factor if the at-risk population is considered as one homogeneous, freely mixing group. Thus either gonorrhea must currently be far from equilibrium and its prevalence rapidly growing in the United States (which does not appear to be the case), or else we must, in YHN's words, "separate the population at risk for gonorrhea into many subgroups, by dividing according to sex, age, race, sexual practices, number of sex partners and other relevant factors". These authors argue, for example, that a 'core' of around half-a-million people having high prevalence (of the order of 20 per cent) would have substantial preemptive effects, and could account for the observed data. Essentially what is happening is that each infective individual in the core population, on average, infects more than one susceptible individual during the course of his infection, while each infective individual outside the core, on average, infects less than one susceptible; at equilibrium, these two rates combine to produce an overall average of one new infectee for each current infection.

The existence of such a core of very sexually active transmitters has immediate implications for the design of control programmes. If the core individuals could all be identified and kept free of gonorrhea (for example, by use of some hypothetical vaccine), the disease would die out of the population, because its basic reproductive rate in the remaining non-core population is less than unity.

In Hethcote, Yorke and Nold's most recent work⁷, they subdivide the population according to sex, the level of sexual activity among the at-risk population (core and non-core) and whether the infections are symptomatic or asymptomatic. A set of eight differential equations is used to model the dynamic interplay between these groups and the consequences of various kinds of control strategies are explored. In these models, the probability of transmission during a sexual intercourse from an infectious female to a susceptible male is estimated⁸ to be 0.2–0.3, while the corresponding

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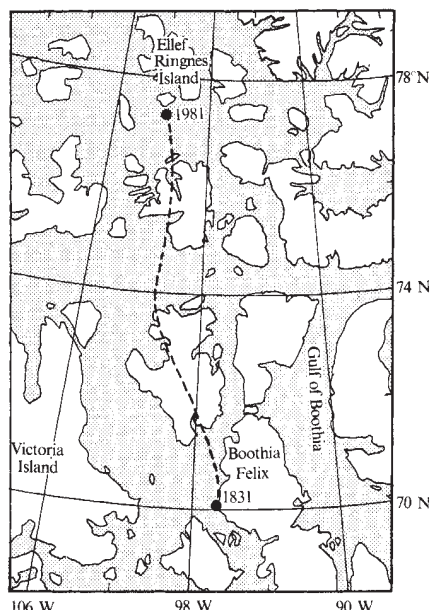
150 years of the North Magnetic Pole

from David R. Barraclough and S. R. C. Malin

ON June 1st, 1831, Commander James Clark Ross "fixed the British flag on the spot, and took possession of the North Magnetic Pole and its adjoining territory, in the name of Great Britain and King William the Fourth"¹. The spot referred to was at 70° 5' 17" N, 96° 46' 45" W where the mean value of magnetic dip, observed over nearly 24 hours, was 89° 59'. This was, as nearly as could be determined with the instruments then available, the position of the North Magnetic Pole (where the dip would be exactly 90°). Commander Ross thus became the first man knowingly to set foot on (there was an eskimo settlement nearby) the North Magnetic Pole.

James Clark Ross was not a stranger to Arctic exploration. After entering the Royal Navy at the age of 12 he served under his uncle, John Ross, for six years. In 1818 he was a member of the expedition, led by John Ross, which began the series of nineteenth century assaults on the North-West Passage. For the next nine years the younger Ross was almost continuously in the Arctic, serving under Parry on his four famous expeditions.

In 1829 John Ross organized an expedition, financed by Felix Booth, the gin distiller, to make yet another attempt to find the North-West Passage. As his second-in-command he chose his by now very experienced nephew. It is not clear how importantly the discovery of the position of the North Magnetic Pole figured in the original plans for the



Path of the North Magnetic Pole, 1831—1981.

expedition, but it was ultimately to prove the major achievement, since the attempt to find the North-West Passage failed. In fact, having spent the first winter frozen in on the east coast of Boothia Felix (now the Boothia Peninsula), the expedition ship *Victory* was able to move only about 8 km northwards during the next two years in an unsuccessful attempt to escape from what had proved to be a dead end. During this enforced stay in Boothia Felix, the younger Ross led several sledging expeditions across and along the peninsula. He also made a

series of geomagnetic observations from which he estimated the probable position of the North Magnetic Pole to be within range of a sledging party. Accordingly, during the last summer in Boothia, James Clark Ross set out to discover the Pole's position and succeeded in doing so.

The story of how the expedition finally extricated itself and was almost miraculously rescued after a fourth winter in the Canadian Arctic cannot be told here. John Ross was knighted in recognition of his achievements and his nephew became the acknowledged leader in the field of polar exploration. James Clark Ross was himself awarded a knighthood on the return of his successful Antarctic expedition during which he came close to, but was unable to reach, the South Magnetic Pole.

The North Magnetic Pole has moved about 800 km north during the 150 years since its discovery, as indicated in the figure. Its present position² is about 77.3°N, 101.8°W.

Sir James Clark Ross's achievement, together with those of other British geomagneticians, is being marked in an exhibition 'The Earth is a Magnet' to be held in the Royal Scottish Museum, Edinburgh, from June 8 until the end of August.

1. Report of Commander, now Captain, James Clark Ross, RN, FRS, FLS in *Narrative of a Second Voyage in Search of a North-West Passage, and of a Residence in the Arctic Regions during the years 1829, 1830, 1831, 1832, 1833* (Webster, London, 1835).
2. *Magnetic Declination Chart 1980.0*, Canada (Division of Geomagnetism, Department of Energy, Mines and Resources, Ottawa).

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probability of transmission from an infectious male to a susceptible female is around 0.5–0.7; since a new sexual encounter typically involves more than one sexual intercourse, the net female-to-male transmission probability may be around 0.5, and the male-to-female around 0.9, per encounter. These detailed values are of less significance in the models than is their ratio, namely around 1:2 (female-to-male versus male-to-female).

Broadly, Hethcote, Yorke and Nold⁷ consider three control strategies. Strategy AW involves the general screening of females, by testing cultures taken from women who use certain designated health facilities. The corresponding strategy AM does the same thing for males. Strategies BW and BM trace females and males, respectively, to whom an infection has been spread by a diagnosed infected individual. Strategy CW identifies infectious females by the men they have infected; strategy CM correspondingly identifies infectious males by their women infectees. In short, strategy A takes a slice

in time, B pursues the chain downstream, and C pursues it upstream.

Although the quantitative details depend on the specific parameters chosen in the various models, strategy C is always more effective than A, which in turn is markedly superior to B. YHN explain this by observing that asymptomatics are more important transmitters than symptomatics because they are infectious for a longer time, and that core persons are more important transmitters because they contact more people while they are infectious. Strategy C has a high likelihood both of identifying very active core individuals and of identifying asymptomatics; strategy A, although relatively ineffective in locating core people, tends to find asymptomatics; while strategy B is not particularly effective in finding either core individuals or asymptomatics. Within any one strategy, finding infectious males by M controls is generally at least as effective at reducing prevalence levels as is finding infectious females by W controls.

Hethcote, Yorke and Nold⁷ sensibly

conclude by stressing that "the relative merits of the various control procedures depend not only on the effectiveness per discovery in reducing prevalence, but also on the cost of discovering an infective by the procedures". If field studies can be designed to evaluate the cost in dollars, or at least the relative costs, for the discovery and cure of one person by each of the general strategies, the way will be opened to the rational design of 'cost effective' programmes. "Different cities or states might come to different conclusions based on the differing nature of their populations".

1. *United States Morbidity and Mortality Weekly Report* 28, 605 (1980).
2. Cooke, K. L. & Yorke, J. A. *Math. Biosci.* 16, 75 (1973).
3. Reynolds, G. H. & Chan, Y. K. *Bull. Inst. int. Statist.* 106, 264 (1975).
4. Hethcote, W. H. *Math. Biosci.* 28, 335 (1976).
5. Constable, G. M. *Bull. Inst. int. Statist.* 106, 256 (1975).
6. Yorke, J. A., Hethcote, H. W. & Nold, A. *Sex. Trans. Dis.* 5, 51 (1978).
7. Hethcote, H. W., Yorke, H. A. & Nold, A. *Math. Biosci.* (in the press).
8. Wiesner, P. J. & Thompson, S. E. *Disease-a-Month* 27 (5), 1 (Feb., 1980).

The treatment of inborn errors of the urea cycle

from Isabel Smith

THE UREA CYCLE is the means by which mammals remove excess ammonia from the body. Every enzyme in the cycle (see the figure) is subject to inherited abnormality, thus giving rise to a group of rare diseases known as the urea cycle disorders¹. With the exception of ornithine carbamyl phosphate transferase (OCT) deficiency, which is a sex-linked condition, the disorders appear to be inherited recessively. They are characterized by a tendency to accumulate ammonia, which may be severe enough to cause death within a few days of birth, or so mild that it is present only after a protein meal and remains symptomless throughout life.

Typical symptoms of ammonia intoxication include recurrent attacks of vomiting, lethargy and drowsiness, often precipitated by catabolic events such as infection, or by increased protein intake. Severely affected patients commonly progress to coma and death, often in the neonatal period. Mental retardation, epilepsy and other neurological abnormalities are frequent in the survivors. Levin and co-workers²

reported beneficial effects of a reduced protein intake on heterozygous, female carriers of OCT deficiency, and since then a low-protein diet supplemented with extra fat and carbohydrate has formed a basis for treatment. Unfortunately these measures alone fail to prevent ammonia intoxication in patients with more severe types of illness, and in growing children prolonged use of a low-protein diet may cause deficiency of essential amino acids and thereby exacerbate nutritional problems arising from the feeding difficulties caused by hyperammonaemia.

Recent advances in therapy have come through a better understanding of the special nutritional needs of patients with urea cycle disorders, and through exploitation of improved methods of reducing intake, and increasing utilization and excretion, of excess nitrogen³⁻⁷. Life-threatening episodes of hyperam-

monaemia can be treated with peritoneal or haemodialysis before irreversible brain damage occurs^{8,9}. In addition, it has been realized that not all symptoms are due to ammonia intoxication. Patients with arginase deficiency, for example, become severely retarded and have limb spasticity although they exhibit only slight hyperammonaemia. Their neurological abnormalities appear to be caused by arginine accumulation. A patient whose blood arginine levels were reduced by means of a low-arginine diet, which was started in the neonatal period, made normal intellectual and neurological development, in striking contrast to the typical symptoms present in an older untreated sibling¹⁰.

Arginine has also become important in the treatment of other urea cycle disorders. It is the only amino acid of the cycle to be incorporated into protein. Normally it is formed in the cycle and is non-essential, but when a block occurs, an exogenous supply of arginine is needed for protein synthesis. In disorders other than arginase deficiency plasma arginine levels are low,

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100 years ago

A SINGULAR CASE OF SHIPWRECK

THE wreck of the Danish mail steamer *Phoenix* which took place off the west coast of Iceland on January 29, was attended by rather unusual circumstances deserving of note. The vessel (about 450 tons burden) sailed with cargo and the mails from Copenhagen for Leith, the Farøe Islands, and Iceland, about the middle of January.

Nothing particular occurred until after leaving the Farøes, when she ran into a severe gale, which rapidly increased to a perfect hurricane, while at the same time the temperature fell to about 50°F. of frost (—18°F.). Such cold is not extraordinary in these latitudes in winter but fortunately it is seldom associated with very high winds. Under the circumstances in which the *Phoenix* was placed every sea that she shipped froze, and the deck soon became covered with a foot or two of solid ice.

As time passed on the continued action of the sea raised a perfect iceberg on the forward part of the vessel, while the showers of spray carried along by the steadily increasing gale covered the masts, yards, and rigging with an ever-thickening coating of ice. Two or three days passed without the least abatement of the storm, and then the half-smothered steamer went over on her beam ends. The crew succeeded in cutting away the masts, and she

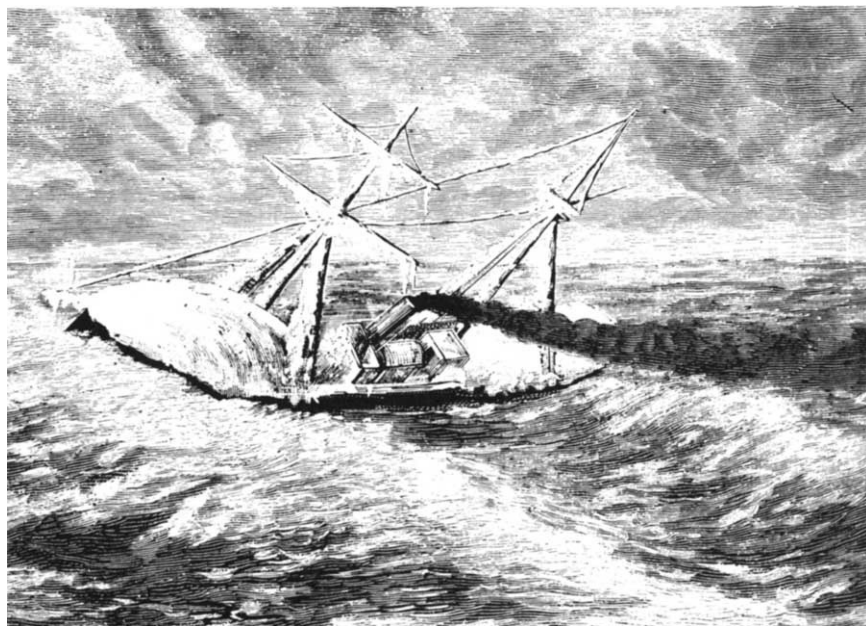
once more righted.

It however was clear, the gale showing no signs of breaking, that the relief was only temporary. The ice continued to form on the vessel, particularly about the fore-castle, where, piled high above the bulwarks, and overhanging the sides, it threatened, by altering her trim, to raise the propeller out of the water.

Under these circumstances, on the morning of January 29, Capt. Kihl decided to run the steamer ashore while daylight lasted. At some distance from the land she struck on a sunken rock, and the crew, taking to the boats, only succeeded with the greatest difficulty in reaching the shore, saving nothing but their lives, the English mail, and a bundle of

blankets which (when carried ashore) was found to be useless — frozen into a solid lump. Hardly one escaped more or less injury from the effects of the extreme cold to which they had been so long exposed. Capt. Kihl and the bulk of his crew soon after succeeded in getting to Reykjavik, and on April 13 they sailed in the sister steamer, the *Arcturus*, for Copenhagen. The officers and men of the wrecked vessel are of opinion that had Capt. Kihl not decided on the 29th to run the *Phoenix* ashore in daylight not a soul would have been saved, as the gale did not moderate for several days after; and the steamer, buried as it was under an enormous mass of ice, must have foundered in the night.

from *Nature* 24, 2 June, 106, 1881

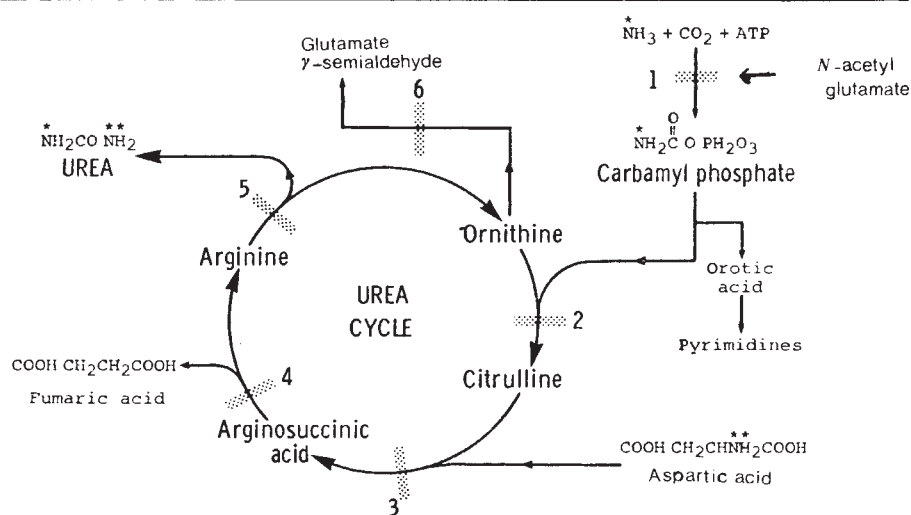


contributing to growth impairment. A low-protein diet given without arginine supplements increases the tendency to arginine deficiency. Poor growth from any cause reduces utilization of nitrogen, thereby tending to increase ammonia accumulation. In arginosuccinic acid lyase (ASL)⁶ deficiencies, lack of arginine causes not only growth impairment but also, by reducing the supply of ornithine (as discussed in detail below), contributes significantly to persistence of raised ammonia levels.

The role of arginine in ammonia homeostasis arises because it is the main precursor of ornithine which is required for uptake of carbamyl phosphate¹¹. The potentially reversible ornithine amino transferase (OAT) step seems normally to proceed towards glutamate semialdehyde. In experimental animals arginine deficiency causes decreased intracellular ornithine levels and hyperammonaemia, even when the urea cycle itself is intact¹¹. A similar situation arises in human patients with lysinuric protein intolerance, which is due to an inherited defect in the transport of lysine out of the cells of small-bowel mucosa, liver and renal tubules¹². Lysine accumulates intracellularly leading to competitive inhibition of arginine and ornithine uptake and to intracellular ornithine deficiency. Administration of arginine intravenously rapidly abolishes the hyperammonaemia¹³, and oral supplements of arginine, or ornithine, or, best of all, citrulline (because uptake of this amino acid is not inhibited by lysine), maintain normal ammonia levels over long periods when given in combination with a low-protein diet¹².

Although not, strictly speaking, urea cycle disorders, two different inborn errors of ornithine metabolism, both of which lead to high plasma levels of ornithine, illustrate relevant points about the role of arginine in ammonia homeostasis. One, OAT deficiency¹⁴, is accompanied by progressive gyrate atrophy of the choroid and retina starting during the first or second decade of life. Patients are of normal intelligence. Plasma levels of ammonia, and its precursors glutamine and glutamic acid, are abnormally low probably because intracellular levels of ornithine, like plasma levels, are high. Treatment with a low-arginine diet restores normal plasma levels of ornithine, ammonia, glutamine and glutamic acid, and there is some evidence that progression of visual abnormalities is halted. The other disorder, which seems to be due to a defect in the transport of ornithine into mitochondria¹⁵, is accompanied by severe mental retardation and marked hyperammonaemia, probably due to intracellular deficiency of ornithine in spite of the elevated plasma levels. As might be expected of such a disorder, patients derive no benefit from a diet which lowers plasma ornithine levels.

Lowered intracellular ornithine levels



1, carbamyl phosphate synthetase (CPS). 2, ornithine carbamyl phosphate transferase (OCT). 3, arginosuccinic acid synthetase (ASS). 4, arginosuccinic acid lyase (ASL). 5, arginase. 6, ornithine aminotransferase (OAT). *One nitrogen atom enters the cycle at step 1; **one nitrogen atom enters the cycle at step 3. Reactions 1, 2 and 6 take place within the mitochondria, 3, 4 and 5 in the cytosol.

are likely to play a major part in the production of hyperammonaemia in arginase deficiency and in ASS and ASL deficiencies. From a study of an infant with ASS deficiency (also called citrullinaemia), Walser *et al.*⁴ estimated that a minimum arginine supplement of 2.2 mmol per kg per day was needed to provide sufficient ornithine for carbamyl phosphate uptake and to allow for normal growth. Arginine supplements combined with a natural protein intake of 1.7 g per kg per day (just at the lower limit of normal for infants) maintained normal ammonia levels for several months; development proceeded normally. The infant died suddenly of hyperammonaemia, however, during an infection at the age of seven months.

An even more impressive effect of arginine supplements (2–5 mmol per kg per day) has been described in patients with ASL deficiency⁶. In this disorder both nitrogen atoms can enter the cycle normally, and be excreted as arginosuccinic acid, providing sufficient arginine is available (see the figure). Ammonia levels can be maintained in the normal range when 2 g protein per kg per day are given with arginine supplements. With smaller supplements of arginine¹⁶, normal growth was maintained but control of ammonia levels was inadequate and the patient showed slow mental development.

Bachmann and Colombo have noted the excretion of large amounts of orotic acid (a derivative of carbamyl phosphate which is produced in increased amounts when the urea cycle is blocked beyond CPS, see figure) in the urine of patients with arginase deficiency, in spite of the mild degree of ammonia accumulation. In ASS and ASL deficiencies, in which ammonia levels are higher, much less orotic acid is excreted. These findings suggest that production of carbamyl phosphate may be increased in arginase deficiency and Bachmann and Colombo have proposed that this could be

due to stimulation, by high arginine levels, of *N*-acetylglutamate synthetase, an enzyme responsible for production of *N*-acetylglutamate, which is the essential co-factor for CPS. Activity of CPS is directly proportional to the concentration of co-factor¹⁸. There is some *in vitro* evidence that arginine does increase *N*-acetyl synthetase activity¹⁷. If the hypothesis is correct, arginine deficiency could reduce ammonia disposal by reducing CPS activity, as well as by the mechanism discussed above, and arginine supplements might thus improve control of ammonia levels by stimulating CPS in OCT, ASS and ASL deficiencies and in partial defects of CPS.

Whatever the mechanisms involved, arginine supplements added to a reduced protein intake benefit many patients with urea cycle defects. In severe forms of CPS, OCT and ASS deficiencies, however, additional measures are needed. One approach is to replace natural protein by a mixture of essential L-amino acids³. This allows nitrogen intake to be reduced, stimulates nitrogen utilization for synthesis of non-essential amino acids, and ensures that intake of essential amino acid requirements for growth are met. Another similar approach, using a mixture of keto-acid analogues of essential amino acids¹⁹, reduces intake, and stimulates utilization, of nitrogen even further. As threonine and lysine cannot be synthesized *in vivo* by transamination, and as the keto-acid derivatives of certain other amino acids are in short supply, a mixture of keto-acid analogues (leucine, isoleucine, valine, phenylalanine and methionine) is given with a mixture of essential L-amino acids (threonine, lysine, tryptophan, histidine), with arginine, to replace natural protein^{20,21}. Patients with severe CPS, OCT and ASS deficiencies, which have been treated in one of these ways, have remained healthy, with good control of

ammonia levels, for many months.

A very different approach to the control of ammonia levels has been introduced by Brusilow *et al.*^{7,22}. Sodium benzoate and phenylacetic acid are excreted in urine after conjugation with the nitrogen-containing compounds glycine and glutamine respectively. A patient with partial CPS deficiency was given 6 g sodium benzoate, and then 6 g of phenylacetate. On each occasion, urinary nitrogen excretion increased by approximately 50 per cent and blood ammonia levels (which were within the normal range whilst the patient was receiving a low-protein diet) and glutamine levels fell. Four patients in coma due to ammonia intoxication were given 200–350 mg per kg of sodium benzoate intravenously and again blood ammonia levels fell, this time from 100 times normal to near normal.

It seems that with a combination of therapeutic strategies long-term treatment of the severe forms of urea cycle disorder is becoming possible²³. Nevertheless, many patients remain at risk of sudden onset of hyperammonaemic coma, due to infection or dietary indiscretion, and the incidence of sudden death remains high. Prompt treatment of infections and avoidance of catabolism by ensuring an adequate energy intake at all times is an important element of long-term treatment²⁴. Peritoneal or haemodialysis, in skilled hands, effectively removes excess ammonia when other measures fail but these procedures need to be started before irreversible brain damage has occurred. The demands which these advances in treatment make on medical services and the families involved are great, and much more information is needed about long-term survival, intellectual progress and social adjustment before the real impact of these developments can be assessed. □

Late-glacial climatic changes

from Peter Moore

CONSIDERABLE interest and controversy surround the climatic events at the close of the last glaciation. In north-west Europe, the warming which began at around 13,500 BP was interrupted by one major cold phase (at about 11,000–10,000 BP) and one less severe event (at about 12,000–11,800 BP) (dates according to Mangerud *et al.* *Boreas* 3; 109, 1974). Both of these cold episodes are clearly expressed in the physical nature of lake sediments and in the contained pollen assemblages of sites in mainland north-west Europe, such as Denmark; but the earlier cold phase is not well marked either sedimentologically or palynologically in British sites, especially western ones. This has led Pennington (*Boreas* 4; 157, 1975) to define a single 'Windermere' interstadial for the British Isles, effectively encompassing the time covered by the two continental interstadials.

Moving further east and south across Europe, one finds that the evidence for this complex of climatic oscillations is weaker, and the amplitude of the climatic fluctuations is evidently dampened. Watts (in *Studies in the Late-Glacial of North-West Europe* (eds J.J. Lowe, J.M. Gray & J.E. Robinson) Pergamon Press, Oxford; 1980) claims that there is no Alpine site which clearly supports a double oscillation of climate in the late-glacial and that even the major cold phase (the 'Younger Dryas' of northern Europe) may be difficult to define, though it is often marked by an opening of *Pinus cembra* and *Larix* forest and an invasion of *Juniperus* and *Artemisia*. Soil erosion at the time may also be reflected in an increased input of minerogenic sediments into lake basins. Data from the Mediterranean area and south-west Europe for the late-glacial period are still rather scanty and open to a variety of interpretations.

On the other side of the Atlantic, there have been pollen diagrams from North America which suggest a late-glacial (Wisconsin) climatic oscillation. Perhaps the best known of these is that of Schweger (*Ecology* 50; 859, 1969) in which a *Picea* and *Fraxinus*-dominated early stage of forest development is interrupted by a period in which the arboreal/non-arboreal pollen ratio falls and *Artemisia* increase. The warm phase, dated at 11,640 BP, has been termed the 'Two Creeks Interstadial' and geomorphological evidence shows that there were minor extensions and contractions of the ice sheet at the closing stages of the Wisconsin glaciation (Wright *Geol. Soc. Am. Mem.* 136; 251, 1973). Some of the most detailed pollen data for this period have come from the work of Birks at Wolf Creek in Minnesota (*Ecol. Monogr.* 46; 395, 1976). He has

demonstrated the existence, between 20,500 and 14,700 BP, of tundra vegetation which was invaded by dwarf shrub with willow and alder, and then at about 13,600 BP, *Picea* became the dominant pollen type. Between 13,600 and 10,000 BP there was no evidence for substantial change in the vegetation, but invasion by *Pinus* subsequently followed. So, the climatic changes which resulted in movement of the residual ice front over that late-Wisconsin time period in Minnesota did not cause marked fluctuations in the vegetation around Wolf Creek. Birks considers it likely that the climatic thresholds necessary to cause such changes were not crossed. The inertia and inherent stability of the spruce forest evidently withstood the climatic oscillations, though their effect seems to have been felt more evidently in Schweger's site at Iola Bog, Wisconsin.

In the other direction, a pollen diagram from the Peking district of China (Kong and Du *Acta bot. sinica* 22; 330, 1980), covering the period of about 30,000–10,000 BP, permits an examination of late-glacial events in central Asia. In the basal sediments (older than 22,700 BP) *Artemisia*, *Chenopodiaceae* and *Gramineae* dominate, indicating a cold, arid steppe-tundra. At about 13,000 BP *Abies*, *Picea* and *Pinus* expand and *Artemisia* contracts, as gymnosperm-dominated forests invaded the Peking plain. By 11,850 *Tilia* (a taxon demanding warmth) begins to expand, and the authors suggest this point as the base of the 'Holocene'. But before a radiocarbon date of 10,750 BP, there are two marked peaks in the pollen of *Artemisia*, the first being accompanied by *Thalictrum* and the second by *Chenopodiaceae*. It is tempting to interpret these as times of reversal in the trend towards warmer climate, permitting once again the temporary extension of the steppe-tundra herbs.

The late-glacial climatic oscillation is not a European anomaly, as was once suggested by Mercer (*Arctic & Alpine Res.* 1; 227, 1969), though it does seem to be both more marked and more consistently recorded in north-west Europe than elsewhere. This may, in part, be due to the current paucity of information from many areas of southern Europe and Asia. Attempts to correlate warm and cold episodes over wide geographical areas are frustrated by the complexity of sequences, local variations in the record (perhaps a consequence of the threshold problem) and, not least, by the inherent errors in the radiocarbon dating of sediments. □

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- Shih, E. in *The Metabolic Basis of Inherited Disease* (eds Stanbury, J.B., Wyngaarden, J. B. & Fredrickson, D. S.) 362 (McGraw Hill, New York, 1978).
- Levin, B. & Russell, A. *Am. J. Dis. Child* 113, 142 (1967).
- Snyderman, S. E. *et al. Pediatrics* 56, 65 (1976).
- Walser, M. *et al. Clin. Sci. molec. Med.* 53, 173 (1977).
- Batshaw, M. L., Brusilow, S. & Walser, M. *Pediatrics* 58, 227 (1976).
- Brusilow, S. W. & Batshaw, M. L. *Lancet* i, 134 (1979).
- Brusilow, S. W., Valle, D. L. & Batshaw, M. L. *Lancet* ii, 452 (1979).
- Batshaw, M. L. & Brusilow, S. W. *J. Pediatr.* 97, 893 (1980).
- Wiegand, C. *et al. J. Pediatr.* 96, 142 (1980).
- Snyderman, S. E. *et al. J. Pediatr.* 95, 61 (1979).
- Visek, W. J. *Nutr. Rev.* 37, 273 (1979).
- Rajantie, F., Simell, O. & Perheentupa, J. *Lancet* i, 1219 (1980).
- Oyanagi, K. *et al. J. Pediatr.* 94, 255 (1979).
- Valle, D., Walser, M. & Brusilow, S. W. *J. clin. Invest.* 65, 371 (1980).
- Hommes, F. A. *et al. Rep. int. Symp. on Metabolic Disease, Interlaken* (1980).
- Collins, F. S. *et al. J. Pediatr.* 96, 429 (1980).
- Bachmann, C. & Colombo, J. P. *Eur. J. Pediatr.* 134, 109 (1980).
- Stewart, P. M. & Walser, M. *J. biol. Chem.* 255, 5270 (1980).
- Close, J. H. *New Engl. J. Med.* 290, 663 (1974).
- McReynolds, J. W. *et al. J. Pediatr.* 93, 421 (1978).
- Thoenes, S. *et al. J. Pediatr.* 90, 213 (1977).
- Brusilow, S., Tinker, J. & Batshaw, M. L. *Lancet* ii, 432 (1979).
- Brusilow, S., Thomas, G. H. & Brusilow, S. W. *Pap. at Meet. on Inborn Errors of Metabolism*, Florida (1980).
- Smith, I. & Francis, D. E. M. in *Textbook of Paediatric Nutrition* (eds McLaren, D. & Burman, D.) 273 (Churchill Livingstone, 1976).

REVIEW ARTICLE

The evolution of metabolic cycles

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The function of the citric acid cycle is to convert efficiently the energy released during the combustion of acetate into the energy stored in the pyrophosphate bonds of ATP. The cycle is almost twice as efficient as feasible alternatives of acetate combustion, such as a direct pathway via glycollate, glyoxylate, formaldehyde and formate. The reason is that the first stage of acetate degradation cannot be a dehydrogenation; it must be an oxygenation by molecular oxygen. Thus the cycle must have evolved because in a competitive environment the chances of survival are greatest if resources are optimal. Analogous considerations apply to the evolution of other metabolic cycles.

WHY have metabolic cycles, such as the citric acid cycle, arisen in the course of evolution? The starting point of the explanation presented here is a basic principle of evolution by natural selection which states that in a competitive environment, the chances of survival are greatest if optimal use is made of resources; or, as Orgel and Crick¹ have recently formulated it, replacing survival of the individual by survival of the species: the theory of natural selection in its more general formulation deals with the competition between replicating entities. In such a competition, the more efficient replicators increase in numbers at the expense of their less efficient competitors. After a sufficient time, only the most efficient replicators survive.

Definition of metabolic cycles

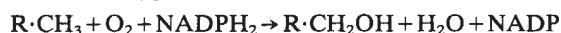
This discussion is limited, in the first instance, to 'metabolic cycles', defined as processes in which an overall chemical change is brought about by a cyclic reaction sequence. The citric acid cycle is a prototype: the overall change is the complete combustion of acetate, achieved by the cyclic formation of di- and tricarboxylic acids. Other types of cyclic processes exist where no overall change occurs, such as the storage cycles of glycogen or of triglyceride. In times of plenty, glucose is converted to glycogen, and in times of need, the glycogen is reconverted into glucose. Similarly, triglyceride is deposited when there is an excess source of energy and it is degraded by hydrolysis when more energy is needed than is supplied by the food.

The evolution of the citric acid cycle

The evolutionary axiom defined above leads to the question: does the citric acid cycle possess advantages over other pathways of acetate oxidation? It is the function of this cycle to provide energy in a form that can be utilized by living cells, that is, in the form of ATP. Hence the most advantageous mechanism of acetate oxidation is that which gives the highest yield of ATP. In evaluating the potential yield of ATP it has to be borne in mind that the bulk of ATP synthesis is not directly connected with the degradation of the foodstuff, but with the transport of hydrogen atoms (or electrons) from NADH to molecular oxygen. Thus it is a prerequisite of ATP synthesis that the primary process of degradation is a dehydrogenation.

The question has been considered whether a direct oxidation of acetate via glycollate, glyoxylate, formaldehyde and formate may be an alternative to the citric acid cycle². An alternative possibility is an initial isomerization of acetic acid to glycolaldehyde, followed by the formation of glycollate. However, calculation of the equilibrium constant for this isomerization from standard heats of formation indicates that glycolaldehyde is about 76 kcal mol⁻¹ less stable than acetic acid. Such a highly endothermic pathway must be ruled out. In the past no answer could be given to the question of why in living matter acetate is not 'directly' oxidized via glycollate. We now wish to offer an answer: the conversion of acetate or acetyl coenzyme A into glycollate cannot be brought about by a dehydrogenation. This

reaction therefore cannot donate electrons to the mitochondrial electron transport chain. It can be oxidized only by molecular oxygen through monooxygenase ('hydroxylase' or 'mixed function oxidase'). This enzyme system, which is not located in the mitochondria but in the microsomal fraction, catalyses the insertion of one oxygen atom of molecular oxygen into the organic substrate while the second oxygen atom is reduced to water. The reduction requires a second substrate that donates the electrons; in most cases this is NADPH₂. The general equation of an oxygenase reaction is therefore



where R·CH₃ stands for any methyl group which, because of the nature of R, cannot be dehydrogenated. R can be —H, or —COOH, or, as in the case of toluene or xylene, a benzene ring.

Not only is an oxygenation of acetate wasteful because it cannot be coupled with ATP synthesis, but in addition, the consumption of one NADPH₂ for the direct reduction of the second O atom of O₂ robs the cell of the potential synthesis of three ATP molecules. The quantitative aspects of the waste are as follows: when free acetate is oxidized through the citric acid cycle there is a net gain of 10 pyrophosphate bonds of ATP. Twelve are formed by the reactions of the cycle but two pyrophosphate bonds are used for the conversion of acetate into acetyl coenzyme A.

When carbohydrate is the precursor, acetate arises in the form of acetyl coenzyme A directly from pyruvate. When long-chain fatty acids are the precursor of acetate, two pyrophosphate bonds are needed to form acyl-coenzyme A. Acetyl coenzyme A then arises by β-oxidation without any further expenditure of ATP. Hence, for a C₁₈ saturated fatty acid, on average 2/9 pyrophosphate bonds are spent before acetate enters the cycle, and the ATP yield of the cycle is therefore 12–0.22 or 11.78 pyrophosphate bonds for one turn of the cycle.

Were acetate 'directly' degraded via glycollate, then six ATPs would be prevented by the oxygenase reaction (with its need for NADPH₂) from being formed. If all three stages of dehydrogenation from glycollate to CO₂ were brought about by NAD- or NADP-linked dehydrogenases, then no more than six ATPs could be formed because one NADH₂ is needed in the oxygenase reaction. This is a maximum value. The only glycollate-oxidizing enzyme (EC1.3.1.) known is a flavoprotein and therefore can at most yield two ATPs.

This concept does not only explain why the direct oxidation of acetate cannot compete efficiently with another pathway; it also offers an explanation of why this other pathway must be a cyclic one. The principle is this: when a metabolic sequence of reactions cannot occur efficiently by a direct route but necessitates a primary attachment of a substance to another molecule of low molecular weight (acetate to oxaloacetate), then the 'other molecule of low molecular weight' must be regenerated when the metabolic process of the substrate (acetate) has been

completed. This is necessary, because were it not so, the other molecule of low molecular weight would have to be produced afresh. This would be wasteful, if not impossible: if the other molecule of low molecular weight were not regenerated it would yield a by-product in stoichiometric proportions, which would be nonsensical: an adult man on a diet of 2,000 kcal would produce 800 g oxaloacetate in 24 h. Thus a cyclic process is to be postulated as the only rational and economic mechanism for the organization of certain metabolic processes.

Optimal use of resources

The prediction that a cyclic pathway of acetate degradation is more efficient than the linear direct route of acetate oxidation does not postulate the specific details of the citric acid cycle. That acetate happens to react as the thioester of coenzyme A and to condense with oxaloacetate may be explained by the well documented assumption that the pathway leading to citrate and thence to α -oxoglutarate had evolved long before O_2 appeared in the atmosphere, in connection with the synthesis of glutamate. Coenzyme A is assumed to have arisen on the surface of the earth very early, possibly before 'life', together with the chemically closely related nucleic acids and other coenzymes³⁻⁵.

Thus in the evolution of the citric acid cycle use was made of mechanisms already existing in connection with other functions. It is, indeed, a general principle of evolution that multiple use is made of given resources. Amino acids have many other functions apart from being building blocks of proteins. For example glycine is a precursor of creatine, bile acids, porphyrins, glutathione and hippurate; serine is a precursor of ethanolamine and choline, that is of phospholipids, and of acetylcholine. These multiple uses represent specific cases of the principle stated earlier concerning optimal use of resources.

Advantage of additional control points

Apart from being more efficient in making optimal use of the energy released by the combustion of acetate, the citric acid cycle has the advantage over a linear reaction sequence because it offers additional possibilities for metabolic control.

The cycle is regulated at several stages^{6,7}, one being the availability of oxaloacetate, the first step of the cycle. As oxaloacetate reacts like a catalyst, a cyclic organization has the advantage that the faster the rate of the cycle, the more rapid is the regeneration of the catalyst. Thus oxaloacetate is not only a catalyst of the ordinary type promoting a chemical process, but also provides control of a feedback type. Furthermore, the concentration of oxaloacetate can be adjusted by the redox state of the NAD-couple and by the tendency of aspartate aminotransferase to establish an equilibrium state of its substrates.

Other metabolic cycles

Some metabolic cycles are precisely analogous to the citric acid cycle in that the substrate, before it can react, has to combine with another substance of low molecular weight, and this other substance must then be regenerated. Examples are the ornithine cycle of urea synthesis, the Calvin cycle of photosynthesis and the glyoxylate cycle which brings about the synthesis of succinate from molecules of acetate. In the ornithine cycle, the catalyst also provides facilities for introducing a supply of energy in the form of ATP, needed for endothermic synthesis of urea.

The pentose phosphate cycle

Somewhat different in detail, but the same in principle, is the optimal efficiency achieved by the pentose phosphate cycle. The function of the pentose phosphate cycle is to generate $NADPH_2$ for reductive biosyntheses in the cytosol, above all for the synthesis of fat from carbohydrate. $NADPH_2$ is formed by glucose-6-phosphate dehydrogenase plus 6-phosphogluconate dehydrogenase. Thus one molecule of glucose-6-phosphate forms two molecules of $NADPH_2$, with ribulose-5-phosphate as a by-product. A complex series of reactions then reconverts this by-product to glucose-6-phosphate in such a way that six molecules of glucose-6-phosphate entering the cycle are converted to five molecules of glucose-6-phosphate while one undergoes complete degradation to 6 CO_2 and 12 $NADPH_2$. Thus NADP acts in place of O_2 as hydrogen acceptor in this degradation of glucose. The free energy changes in this pathway are slight; hence the pentose phosphate cycle disposes effectively and economically of an unwanted by-product.

The evolution of other metabolic pathways

The question of why certain metabolic pathways have evolved can be profitably raised also about non-cyclic mechanisms. What is the advantage of the pathway of propionate utilization via propionyl coenzyme A, methyl malonate coenzyme A and succinyl coenzyme A, as opposed to primary dehydrogenation to acrylate? What are the advantages of fatty acid synthesis via condensation of acetyl coenzyme A with acyl coenzyme A? Why are ω -oxidation and the desaturation of saturated long-chain fatty acids brought about by monooxygenase, rather than dehydrogenation? A discussion of these questions is beyond the scope of this article.

1. Orgel, L. E. & Crick, F. H. C. *Nature* **284**, 604-607 (1980).
2. Krebs, H. A. *Enzymologia* **12**, 88-100 (1947).
3. Oparin, A. I. *The Origin of Life on the Earth* 3rd edn (Oliver & Boyd, London, 1957).
4. Handler, P. in *Proc. 5th int. Congr. Biochem.* (ed. Oparin, A. I.) 149-157 (Macmillan, New York, 1961).
5. King, G. A. M. *Bio-systems* **13**, 23-45 (1980).
6. Bowman, R. H. *J. biol. Chem.* **241**, 3041-3048. (1966).
7. Randle, P. J., England, P. J. & Denton, R. M. *Biochem. J.* **117**, 677-695 (1970).

ARTICLES

Microwave OH maser emission in the circumstellar envelopes of late-type stars

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The total angular extents and absolute positions (± 1 arc s) of OH maser clouds surrounding 20 red giant and supergiant stars have been measured using the Very Large Array in a spectral line mode. Our results are consistent with a symmetric, expanding circumstellar shell model for many of the sources, but about half of the sources have more complex structure at the arc second level. A correlation is found between the sizes of the masing regions at 1,612 MHz, the expansion velocities of the circumstellar shells, and the estimated mass loss rates. For one star (U Orionis) initiation of extensive mass loss has occurred very recently and dramatic changes in its OH spectrum may represent the 'turn on' stage of OH masers in evolved stars.

MICROWAVE OH maser emission at 1,612 MHz is commonly associated with long-period, variable, oxygen-rich red giant and supergiant stars which are undergoing extensive mass loss. The OH spectra characteristically have a double-peaked velocity structure in which the two strongest spectral features have velocity separations ranging from $\sim 5 \text{ km s}^{-1}$ to 50 km s^{-1} . Evidence that the stellar velocity is the mean velocity of the two OH peaks and that the OH masers can occur at large distances from the star¹⁻⁴ make an expanding, circumstellar shell model particularly attractive for these evolved stars. In this model the low-velocity (blueshifted) emission arises from the near side of the shell (as viewed from the Earth), whereas the high-velocity (redshifted) emission arises from the far side. The terminal expansion velocity V_e of the gas is one half the total velocity separation of the peaks.

Most of our direct knowledge of the structural characteristics of the masing regions associated with these stars is derived from very long baseline interferometric (VLBI) measurements. Unfortunately, such measurements generally provide only lower limits for the extent of the masing regions⁵. In addition, most VLBI maps of the spatial distribution of the maser components for these stars indicate that the distribution is much more complex than is predicted by the simple shell model⁵. Recent results obtained by Booth *et al.*⁶ with relatively short VLBI baselines ($\sim 100 \text{ km}$) for the source OH127.8-0.0 are a dramatic exception, however, and demonstrate that at least for this star the expanding shell model is correct.

To obtain a better understanding of the overall size and structure of the OH masing regions for a significant number of these stars, we have used the National Radio Astronomy Observatory Very Large Array radio telescope near Socorro, New Mexico to obtain lower spatial resolution ($\sim 1.2 \text{ arc s}$ synthesized half-power beamwidth at 18 cm) than is available with VLBI techniques. All observations were made during 22-23 November 1980 with a spectral line mode (frequency resolution = 6.1 kHz after Hanning-smoothing), nine antennas, and right circular polarization. Data from 20 stellar maser sources were obtained with a total integration time of $\sim 1 \text{ h}$ per source. Each maser source was observed at 3 or 4 hour angles, allowing a distribution of observations in the uv plane. Calibrators were observed every 30 min.

We present here only initial results obtained by separately integrating over the total velocity extents of the low-velocity and of the high-velocity emission complexes of each source. Because of the large number of sources which comprise our data, we will

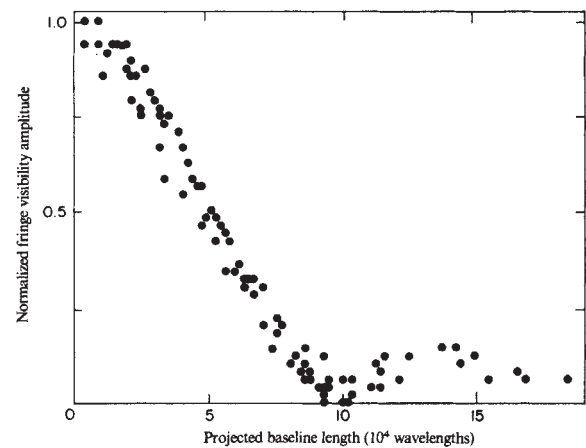


Fig. 1 Normalized fringe visibility as a function of projected baseline length for the integrated high velocity complex ($+52.5$ – $+55.4 \text{ km s}^{-1}$) of R Aquilae. All data for this and subsequent figures were obtained at a rest frequency of 1,612.231 MHz during epoch 1980.9.

present an analysis of the extent and relative positions of individual spectral features and a detailed comparison with shell models elsewhere.

Positions of the maser clouds

Table 1 lists for each source the peak position of the integrated flux density precessed to epoch 1950, the corresponding velocity range for the position determination, the circumstellar expansion velocity derived from total power OH spectra, the estimated distance, the total angular size of the masing region, the corresponding linear size, the estimated mass loss rate $\dot{M}$, and the corresponding reference for $\dot{M}$.

In all cases the velocity range chosen for the position determination was dominated by a relatively strong, narrow spectral component and the resulting maps showed a fairly simple structure with a well-defined maximum intensity. We estimate that the positions listed in Table 1 are accurate to $\pm 1.0 \text{ arc s}$. The relative positions between the high- and low-velocity components are accurate to $\pm 0.3 \text{ arc s}$.

For stars where both the low and high integrated velocity maps showed simple structure, positional offsets between the two complexes were $\leq 0.3 \text{ arc s}$. To within our uncertainties, this

Table 1 Positions, shell sizes, and mass loss rates for OH/IR stars

Star	Position of emission peak			Velocity range (km s ⁻¹)	V _e (kpc)	Distance (arc s)	Angular	Linear	M	Ref.
	α(1950)	δ(1950)	(km s ⁻¹)				size (×10 ³ AU)	size (×10 ⁻⁶ M _⊙ yr ⁻¹)		
IRC+10011	01 h 03 min	48.08s	+12°19′51.2″	− 10.9–− 7.4	18	0.5	2.6×2.2*	1.3	17	7
OH127.8−0.0	01 30	27.63	+62 11 31.2	− 67.5–− 61.2	11	3.3	2.2	7.3	32†	
OH138.0+7.3	03 20	41.48	+65 21 32.8	− 48.8–− 45.4	10	2.4	1.4	3.4	12†	
OH141.7+3.5	03 29	23.66	+60 10 04.4	− 71.0–− 67.6	12	3.7	0.9	3.3	17†	
IRC+50137	05 07	19.68	+52 48 53.7	+ 18.3−+ 21.1	17	0.8	2.4	1.9	11	7
U Ori	05 52	50.93	+20 10 06.1	− 43.5–− 40.1	3	0.2	≤1	≤0.2	1.2	11
IRC+40156	06 29	45.03	+40 45 08.2	− 29.0–− 26.8	12	1.4	1.1	1.5	8†	
VY CMa	07 20	54.72	−25 40 12.5	− 14.6−+ 8.1	28	1.5	2.5×1.4*	3.8	165,600	7,13
IRC−20197	09 42	56.55	−21 47 54.2	+ 26.5−+ 28.8	12	0.7	1.0	0.7	4	7
WX Ser	15 25	31.98	+19 44 13.0	− 1.9−+ 0.9	7	1.0	0.8	0.8	2.8	7,11
OH17.7−2.0	18 27	39.77	−14 31 03.9	+ 48.0−+ 53.6	12	5.4	3.1×1.4*	16.8	88†	
OH26.5+0.6	18 34	52.47	−05 26 37.2	+ 29.3−+ 42.9	14	2.2	4.7×2.2*	10.4	400,70,200	10,12,13
OH25.1−0.3	18 35	33.36	−07 12 35.2	+129.0−+131.8	13	9.1	1.4	12.8	78†	
OH32.8−0.3	18 49	48.15	−00 17 53.2	+ 44.1−+ 47.5	16	4.3	3.2×2.1*	13.8	20,50	10,12
OH39.7+1.5	18 56	03.88	+06 38 49.8	+ 30.2−+ 38.1	16	1.4	2.8×1.8*	3.9	20,50	10,13
OH39.9−0.0	19 01	42.90	+06 08 44.2	+159.3−+165.0	15	7.7	1.6×1.0*	12.3	100	10
R Aql	19 03	57.67	+08 09 07.7	+ 52.5−+ 55.4	6	0.3	3.1	0.9	0.8	11
IRC+10420	19 24	26.74	+11 15 11.2	+ 40.4−+ 63.1	29	3.4	3.3×2.0*	11.2	340†	
RR Aql	19 55	00.30	−02 01 17.5	+ 31.4−+ 34.8	5	0.4	1.0	0.4	0.4†	
NML Cyg	20 44	33.84	+39 55 57.2	− 32.8−+ 1.3	23	0.5	3.8×3.2*	1.9	36,13	7,11

* Indicates complex (multiple-component) structure.

† Values determined from equation (1).

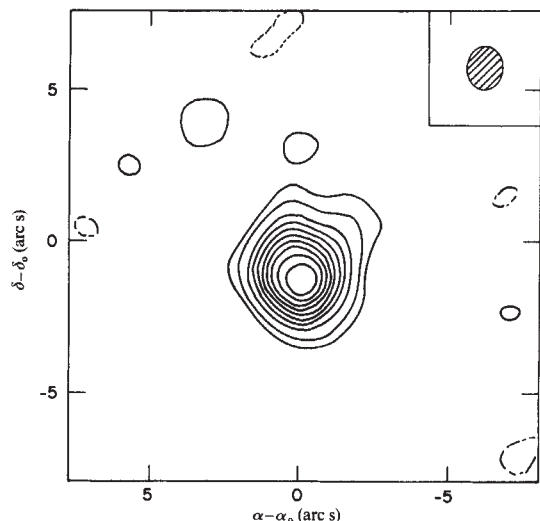


Fig. 2 Angular intensity distribution as a function of position for the integrated high-velocity complex of R Aquilae. Contour levels are in units of -5%, 5%, 10%, 20%, ---, 90% of the peak integrated flux density. The FWHM synthesized beamwidth is shown in the north-west quadrant of the diagram. The map centre is α_0 (1950) = 19 h 03 min 57.68 s, δ_0 (1950) = +08°09'09.1".

result supports a symmetric, expanding shell model for most of the stars. (Our integrated velocity data do not allow us to distinguish between spherical symmetry and other symmetric, expanding geometries.) As this model predicts that the position of maximum maser gain occurs along the line of sight to the star, it is likely in these simple cases that our positions are equivalent to the stellar positions. This view is supported for the bright stars U Ori and R Aql as their positions listed in Table 1 agree with the SAO optical positions to within 1 arc s after correction for the effects of proper motion.

Structure of the maser clouds

Except possibly for U Ori which may be unresolved, all our sources display structure on an arc second scale. About one half of them need to be modelled by more than one spatial component and are classified as complex in Table 1. The large number of sources with simple (single-component) structure may be due to the dominance of the strongest spectral feature in each integrated emission complex.

To illustrate our data for a source with relatively simple structure, we show the visibility function (Fig. 1) and the intensity distribution (Fig. 2) for the high-velocity complex of the Mira variable R Aquilae. The curve in Fig. 1 is a classic visibility function for a circular-symmetric source with a uniform brightness distribution. This result is especially significant as most previous size estimates of stellar maser components assume gaussian brightness distributions. The derived size from Fig. 1 agrees well with the total extent of 3.1 arc s obtained from the map in Fig. 2 after correction for source broadening due to the beam. The slight asymmetry in the emission distribution in Fig. 2 may be an instrumental effect due to inadequate phase calibration for this source. Note, however, that the centroids of the much weaker low velocity complex (not shown) and the high-velocity complex agree in position to within 0.3 arc s, which supports a simple shell model for this source.

As an example of a source with complex structure, Fig. 3 shows the angular distribution of the integrated emission of the low-velocity and high-velocity emission complexes for the M supergiant star NML Cygni. Figure 3 indicates significant differences between the structure of the integrated emission from the two velocity complexes. The differences do not support a simple expanding shell model where the two emission complexes should appear to be spatially coincident at the stellar position. On the other hand, our maps are in very good qualitative agreement with VLBI maps⁵ which show the relative positions of 1,612-MHz maser components towards this star. Points

of similarity include: (1) the central location of the low-velocity emission in the overall masing cloud; (2) positionally separate concentrations of low- and high-velocity emission; and (3) an elongated distribution of the high-velocity emission with a position angle of $\sim 135^\circ$ and a total angular extent of several arc seconds.

Angular sizes of the maser clouds are listed in Table 1. All values were obtained from measurements of the total angular extent of the integrated emission to the lowest significant contour level (typically $\sim 10\%$) and have been corrected for source broadening due to the synthesized beam. In cases where the sizes of the low- and high-velocity complexes are different, we list the larger size. Our size estimates may sometimes be too small because of the limited dynamic range of the maps. For sources with simple structure, however, sizes determined from the maps are in relatively good agreement with sizes determined from the visibility functions if a uniform disk brightness distribution is assumed. We cannot, of course, rule out the possibility of low-level emission ($\leq 10\%$ of the total integrated emission) with much greater scale sizes.

Relation between OH structure and stellar mass loss

We have recently assessed³ the available data pertaining to the structure of OH masing regions in late-type stars and suggested that the overall size of the 1,612-MHz masing region may be related to the stellar mass loss rate. That analysis was hindered, however, by the few OH/IR stars for which measurements of the mass loss rate and the shell size exist. The present data along with recent estimates of the mass loss rates

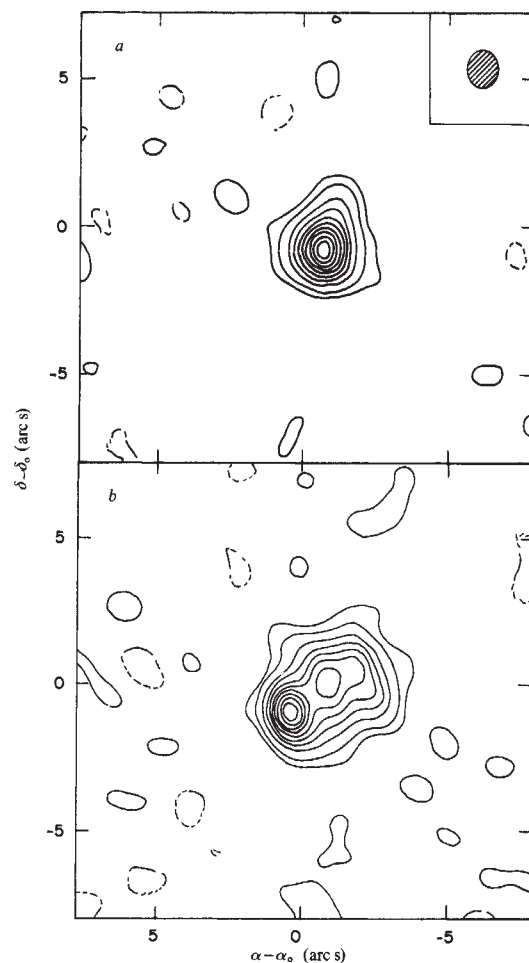


Fig. 3 Angular intensity distribution as a function of position for: *a*, the integrated low-velocity (-32.8 to $+1.3$ km s $^{-1}$); and *b*, the integrated high-velocity ($+2.5$ to $+23.3$ km s $^{-1}$) emission complexes of NML Cygni. Contour levels have the same relative percentages of the peak integrated flux as in Fig. 2. The map centre is α_0 (1950) = 20 h 44 min 33.90 s, δ_0 (1950) = +39°55'58.0".

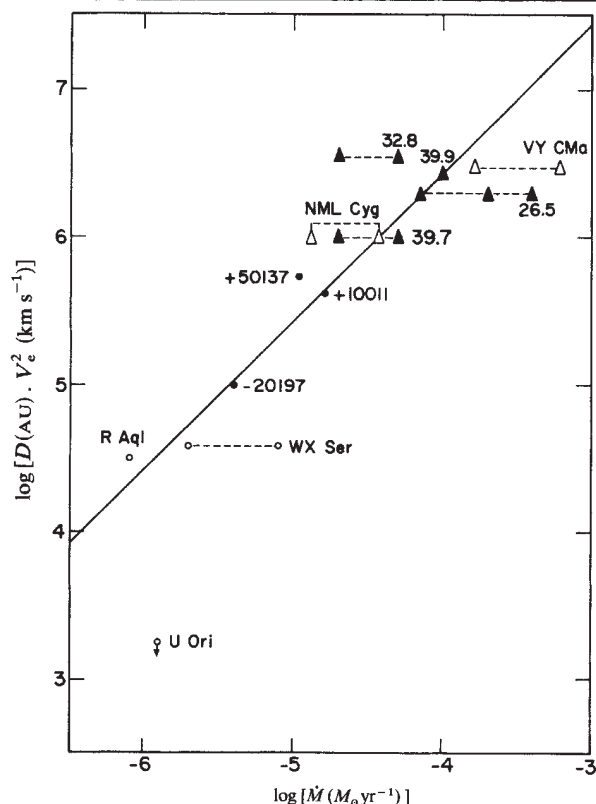


Fig. 4 Product of the total angular extent D (AU) of the 1,612-MHz masing region and the square of the circumstellar expansion velocity V_e (km s^{-1}) as a function of the stellar mass loss rate $\dot{M}$ ($M_\odot \text{ yr}^{-1}$). $\circ$, Optically identified Mira variables; Δ , M supergiants. $\bullet$, Mira variables commonly designated as 'IRC' sources. $\blacktriangle$, Unidentified Type 2 OH/IR stars, designated as 'OH' stars in Table 1. The solid line corresponds to equation (1).

for several additional OH/IR stars now provide a much better data base to test our proposed relation.

To compare the sizes of the masing regions in these stars, we have converted the angular diameters to linear diameters using the distances listed in Table 1. Distances were obtained from ref. 7 for most of the optically identified or 'IRC' stars, from ref. 3 for the 'OH' stars, from ref. 8 for IRC + 10420, NML Cyg, and VY CMa, and a period-luminosity relationship⁹ for U Ori. In general, the distance estimate (and thus the derived linear shell size) is probably only accurate to about a factor of 2.

Estimates of the stellar mass loss rates have been obtained for 12 stars in our sample from measurements of the depth of the 10- μm silicate absorption feature present in the IR spectra of many of these stars^{10,11}, from considerations of momentum conservation in the radiation-transfer process¹², or from model-fitting to the IR excess^{7,13}. For stars observed by Hyland *et al.*⁷, we have derived the total mass loss rate by adopting a canonical value of 100 for the mass ratio of gas to dust in the circumstellar shells. This value is consistent with values found by Werner *et al.*¹² for several OH/IR stars. If more than one estimate of the mass loss rate is available, we have listed all estimates to give an idea of the uncertainties of these derived quantities.

To investigate a possible relationship between the shell diameter D and the stellar mass loss rate $\dot{M}$, we consider a spherically expanding shell with density $n \propto \dot{M}/(r^2 V_e)$ at distance r from the star. Pumping models¹⁴ indicate that the critical

parameter for inversion of the OH rotational transitions is the optical depth τ_{OH} . If the maximum extent of the maser region is determined by the position at which τ_{OH} has a minimum value, we have $\tau_{\text{min}} \propto nr/V_e \propto \dot{M}/(DV_e^2)$. Assuming τ_{min} is constant from star to star, we then have $\dot{M} \propto DV_e^2$, where D and V_e are observables if the distance to the star is known.

The results plotted in Fig. 4 support such a correlation for the stars in Table 1 with values of $\dot{M}$ derived from IR data. A least-squares fit to all the data points in Fig. 4 except U Ori yields $DV_e^2 = 6.8 \times 10^9 \dot{M}^{(0.87 \pm 0.14)}$ with a linear correlation coefficient of 0.90. However, in view of the uncertainties in the data, we have drawn the solid line in Fig. 4 with a slope of 1, corresponding to

$$\log [D(\text{AU}) \cdot V_e^2 (\text{km s}^{-1})] = 10.44 + \log \dot{M} (M_\odot \text{ yr}^{-1}) \quad (1)$$

Equation (1) has been used to compute $\dot{M}$ for the remaining stars in Table 1. As values of $\dot{M}$ obtained from IR measurements depend on various critical assumptions concerning the properties of the dust, there may be considerable errors and the scale of the values for $\dot{M}$ may be uncertain. Nevertheless, the correlation in Fig. 4 suggests that the relative values of $\dot{M}$ for these stars may be approximately correct to an order of magnitude.

The correlation in Fig. 4 also suggests that the expansion time scales of the shells are greater than $D/(2V_e)$ for most of these stars. A prominent exception is U Ori whose size (≈ 200 AU) is the smallest of all the stars we observed (Table 1). This star is particularly noteworthy because of recent dramatic changes in its OH spectrum (from a Type 1 or main-line emitter to a Type 2 or 1,612-MHz emitter)^{15,16} and because of changes in the structure of the OH masing region wherein the apparent sizes of the maser components have increased 10-fold in 3 yr (ref. 17). Such dynamic events have thus far been observed only for this star.

We suggest that initiation of extensive mass loss has occurred fairly recently (≈ 150 yr) for this star. The phenomena observed for the OH masers from U Ori may be manifestations of the physical conditions in the outer regions of a young, expanding envelope whose density and temperature (and thus maser pump conditions) are rapidly changing. The conversion of the OH spectrum from Type 1 to Type 2 is consistent with this model based on theoretical suggestions that the main-line inversion region is inside the 1,612-MHz inversion region¹⁸. The dynamic phenomena observed for this star thus may be representative of the 'turn on' stage of masers associated with late-type stars.

Conclusions

Absolute mean positions (± 1.0 arc s) have been determined for OH maser clouds surrounding 20 red giants and supergiants. In most cases the derived positions are probably the stellar positions.

The structure of the masing regions around many of the stars supports a simple, symmetric, expanding shell model, but there are numerous cases where the structure is significantly more complex.

Diameters of the masing regions vary from $\sim 10^2$ AU to $> 10^4$ AU and are proportional to $\dot{M}/V_e^2$.

Because of the extremely small shell size observed for one star (U Ori) and because of the recent dramatic changes in its OH spectrum, we suggest that the star is surrounded by a young (≈ 150 yr), expanding envelope whose OH masers have only recently been turned on.

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- Dickinson, D. F., Reid, M. J., Morris, M. & Redman, R. *Astrophys. J. Lett.* **220**, L113-L116 (1978).
- Reid, M. J., Muhleman, D. O., Moran, J. M., Johnston, K. J. & Schwartz, P. R. *Astrophys. J.* **214**, 60-77 (1977).
- Bowers, P. F., Reid, M. J., Johnston, K. J., Spencer, J. H. & Moran, J. M. *Astrophys. J.* **242**, 1088-1101 (1980).
- Jewell, P. R., Webber, J. C. & Snyder, L. E. *Astrophys. J. Lett.* **242**, L29-L31 (1980).
- Benson, J. M. & Mutel, R. L. *Astrophys. J.* **233**, 119-126 (1979).
- Booth, R. S., Kus, A. J., Norris, R. P. & Porter, N. D. *Nature* **290**, 382 (1981).
- Hyland, A. R., Becklin, E. E., Frogel, J. A. & Neugebauer, G. *Astr. Astrophys.* **16**, 204-219 (1972).

- Mutel, R. L., Fix, J. D., Benson, J. M. & Webber, J. C. *Astrophys. J.* **228**, 771-779 (1979).
- Clayton, M. L. & Feast, M. W. *Mon. Not. R. astr. Soc.* **146**, 411-421 (1969).
- Mason, S., Gehrz, R. D., Kleinmann, S. G., Hackwell, J. & Grasdalen, G. (in preparation).
- Gehrz, R. D. & Woolf, N. J. *Astrophys. J.* **165**, 285-294 (1971).
- Werner, M. W. *et al. Astrophys. J.* **239**, 540 (1980).
- Telesco, C. M., Kleinmann, S. G., Harper, D. A., Loewenstein, R. & Moseley, S. H. (in preparation).
- Elitzur, M., Goldreich, P. & Scoville, N. *Astrophys. J.* **205**, 384-396 (1976).
- Pataki, L. & Kolena, J. *Bull. Am. astr. Soc.* **6**, 304 (1974).
- Cimernan, M. *Astrophys. J. Lett.* **288**, L79-82 (1979).
- Reid, M. J. *et al. Astrophys. J. Lett.* **227**, L89-92 (1979).
- Elitzur, M. *Astr. Astrophys.* **62**, 305 (1978).

Molecular analysis of the *unc-54* myosin heavy-chain gene of *Caenorhabditis elegans*

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The properties of a small internal deletion mutant, E675, have been exploited in the molecular cloning of the unc-54 gene. This mutation uniquely identifies unc-54 sequences as molecules of altered length in E675 and provides a genetic and physical marker for the active gene, its messenger RNA and its protein product.

THE *unc-54* gene of *Caenorhabditis elegans* specifies a major myosin heavy chain present only in the body wall musculature of this small soil nematode¹⁻³. All mutants in this gene are severely paralysed, and have disorganized muscle in which the numbers of thick filaments are reduced by 75–80% (refs 4, 5). However, these animals are able to feed and survive because the pharyngeal muscle is unaffected and continues to function normally.

A large collection of independently induced mutations in *unc-54* is being analysed in this laboratory as part of a detailed study of this eukaryotic gene. Genetical techniques which allow selection of additional mutants, including deletions, have recently been developed (R. P. Anderson and S. Brenner, unpublished) and several suppressor genes which suppress specific alleles of *unc-54* have been identified^{6,7}.

Many *unc-54* alleles have been characterized by chemical cleavage of purified nematode myosin heavy chains⁴, made possible by the abundance of the body wall muscle contractile proteins and the ease with which they can be isolated from homogenates of the animal. Strains carrying dominant or semi-dominant mutations were found to contain normal amounts of the *unc-54* myosin heavy chain which were unable to assemble into functional thick filaments. Although most of these alleles seem to be missense mutations, in one particular semi-dominant mutant, E675, the *unc-54* myosin heavy chain was reduced in molecular weight (MW) by an internal deletion of 100 amino acids near the C-terminus of the molecule⁸.

Most recessive alleles of *unc-54*, such as *e190*, fail to produce stable protein products and cannot be studied by these methods. The present experiments use cloned complementary DNA and genomic DNA probes to define the structure of the *unc-54* gene, and describe the assay of *unc-54* messenger RNA by cell-free protein synthesis and hybridization. These methods allow detailed study of the recessive alleles of *unc-54*. This is of particular interest because we expect that this class of alleles includes nonsense mutations as well as mutations affecting mRNA synthesis and maturation.

Cell-free synthesis of the *unc-54* myosin heavy chain

RNA was isolated from nematodes using a modification of the guanidinium chloride extraction procedure⁹. This yielded undegraded mRNA which supported high levels of protein synthesis in cell-free systems derived from both wheat-germ¹⁰ and rabbit reticulocytes¹¹.

Total RNA from wild-type animals (N2) programmes the synthesis of three polypeptides of MWs 210,000, 205,000 and 200,000 which have the properties of myosin heavy chains. The putative myosin heavy chains synthesized in the wheat-germ cell-free system were examined by SDS-polyacrylamide gel electrophoresis (Fig. 1). Each of these proteins is quantitatively precipitated by antibody prepared against nematode myosin (purified by affinity chromatography on myosin-Sepharose) and each co-migrates with a heavy-chain component of nematode myosin. A similar result is observed when the reticulocyte cell-free system is programmed with nematode RNA, except that the 210,000-MW component is preferentially synthesized. We therefore worked primarily with the wheat-germ system, in

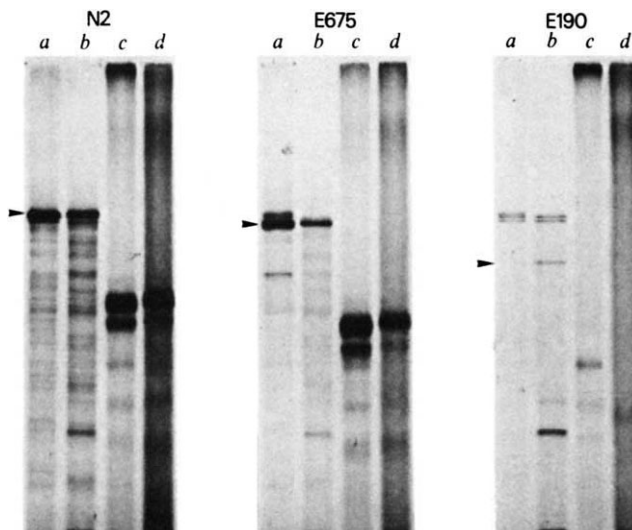


Fig. 1 Cell-free synthesis of nematode myosin heavy chains. RNA was isolated by guanidinium chloride extraction⁹ of nematodes from worms grown in liquid medium³³. The animals were disrupted by two passages through a French pressure cell at 12,000 p.s.i. Cuticular debris was removed by centrifugation at 30,000g for 15 min and RNA selectively precipitated at -20°C for 12–15 h by addition of 0.5 vol 95% ethanol. The precipitated RNA was resuspended in 7 M urea, 0.3 M NaCl, 10 mM Tris-HCl pH 7.4, 1 mM EDTA and 0.2% SDS, and residual protein was removed by three successive phenol/chloroform/isoamyl alcohol extractions (25:24:1). The yield of RNA was usually 5 mg per g wet weight nematodes. Extracts of commercial raw wheat germ (Spillers Research) were prepared as described previously¹⁰ but without preincubation. Protein synthesis was usually performed in a 50- μl reaction mixture containing 15 μl of gel-filtered wheat-germ S-30, 20 μg total nematode RNA, 1 mM ATP, 0.1 mM GTP, 10 mM creatine phosphate, 50 $\mu\text{g ml}^{-1}$ creatine kinase (Boehringer), 20 mM HEPES (adjusted to pH 7.6 with KOH), 1 mM dithiothreitol (DTT), 96 mM KCl, 1.5 mM Mg-acetate, 0.8 mM spermidine, 200 $\mu\text{Ci ml}^{-1}$ ^{35}S -methionine (400–600 Ci mmol⁻¹, Amersham), 5 μM methionine and 40 μM each of the other 19 amino acids. Nematode RNA preparations frequently contained RNA aggregates which inhibited the synthesis of high molecular weight proteins. Incubation of the RNA in 10 mM Tris-HCl pH 7.4 and 0.1 mM EDTA at 65°C for 5 min just before addition to the cell-free system eliminated this problem. Immunoreactive proteins were identified by incubating the wheat-germ reaction mixture with antiserum prepared against nematode myosin and purified by affinity chromatography on myosin-Sepharose. The antigen-antibody complexes were indirectly immunoprecipitated with formaldehyde-fixed *Staphylococcus aureus*³⁴ (MRE, Porton). To obtain sufficient material for analysis of the cyanylation reaction^{8,13,14,35} the standard wheat-germ reaction mixture was increased to 1 ml. The myosin heavy chains were recovered by immunoprecipitation as described previously³⁴ except that the proteins which bound to the immunoabsorbent were solubilized in 6 M guanidinium chloride, 100 mM Tris-HCl pH 8.0 and 10 mM DTT at 37°C for 1 h. Chemical cleavage was performed in the presence of 100 $\mu\text{g ml}^{-1}$ of rabbit skeletal muscle myosin and the cyanylation fragments from the rod portion of the myosin molecule were analysed by SDS-polyacrylamide gel electrophoresis. ^{35}S -labelled myosin was extracted from nematodes grown on ^{35}S -labelled *E. coli*². Gels³⁶ were prepared for fluorography³⁷ and exposed to pre-fogged film³⁸ at -80°C . The autoradiographs depicted above show the myosin heavy chains of N2, E675 and E190 animals. In each panel: a, myosin heavy chains synthesized *in vivo*; b, myosin heavy chains synthesized by the cell-free system and purified by immunoprecipitation; c, cyanylation cleavage of myosin heavy chains synthesized *in vivo*; d, cyanylation cleavage of myosin heavy chains synthesized by the cell-free system. The arrows indicate the mobility of *unc-54*-specific cell-free translation products from each of the three strains.

which the proportions of heavy-chain synthesis more accurately reflect the *in vivo* levels.

The major myosin heavy chain synthesized *in vitro* seems to be the product of translation of *unc-54* mRNA. It co-migrates with the 210,000-MW *unc-54* myosin heavy chain synthesized in wild-type animals, and shows altered patterns of synthesis when the cell-free system is programmed with RNA from *unc-54* mutants. When RNA from E675 animals is used as a source of mRNA, this heavy-chain component is replaced by an equally abundant, but novel, polypeptide of MW 200,000, which now co-migrates with the major myosin heavy chain present in E675 animals. Our previous chemical cleavage studies of E675 myosin demonstrated that the truncated heavy chain arises from an internal deletion of 100 amino acids⁸. As an apparently identical deletion is observed in the E675 myosin heavy chain synthesized *in vitro*, it seems likely that the deletion is present in the *unc-54* mRNA and is not due to post-translational modification.

The wild-type *unc-54* heavy chain is also absent from the translation products of E190 RNA. However, a polypeptide of MW 170,000 can be detected among the products immunoprecipitated from the wheat-germ extract. This component is present in low yield and is found only in E190, suggesting that it may be a product of the *unc-54* mRNA altered by the *e190* mutation. We have failed to detect a polypeptide of similar molecular weight in the immunoprecipitated myosin fractions of E190 animals, probably because the shortened E190 heavy chain fails to assemble correctly into a stable myosin molecule and is therefore subjected to proteolytic degradation. In any case, the low level of *in vitro* synthesis of this product indicates that the *e190* mutation causes defects in either the synthesis or survival of the *unc-54* mRNA.

The other recessive null alleles assayed by cell-free translation also fail to synthesize full-length *unc-54* myosin heavy chain¹². Allele-specific peptides smaller than the wild-type *unc-54* myosin heavy chain have been observed in immunoprecipitates of the translation products of E1092, E1201 and E1223 but not in E1108. The simplest explanation for this is that these peptides are each the products of the mutant *unc-54* mRNAs. However, the low yields of these products have limited their further analysis.

Direct chemical evidence for the synthesis of the *unc-54* myosin heavy chain in the cell-free system was obtained from an analysis of the large polypeptides produced by chemical cleavage of the heavy chains at cysteine residues by

cyanylation^{4,8,13,14}. The major cleavage products of the wild-type *unc-54* myosin heavy chain are two fragments of MWs 150,000 and 140,000 (ref. 4). Cyanylation of the *unc-54* myosin heavy chain from E675 produces an analogous pattern but the large peptides are reduced in molecular weight by 10,000 because of the deletion⁸. This characteristic cyanylation doublet is absent from cleaved E190 myosin heavy chains⁴. As shown in Fig. 1, cleavage of the myosin heavy chains synthesized in the wheat-germ extracts generates the correct cyanylation peptides. This demonstrates the correct placement of the internal cysteine residues and provides conclusive evidence that the *unc-54* myosin heavy chain is synthesized *in vitro*.

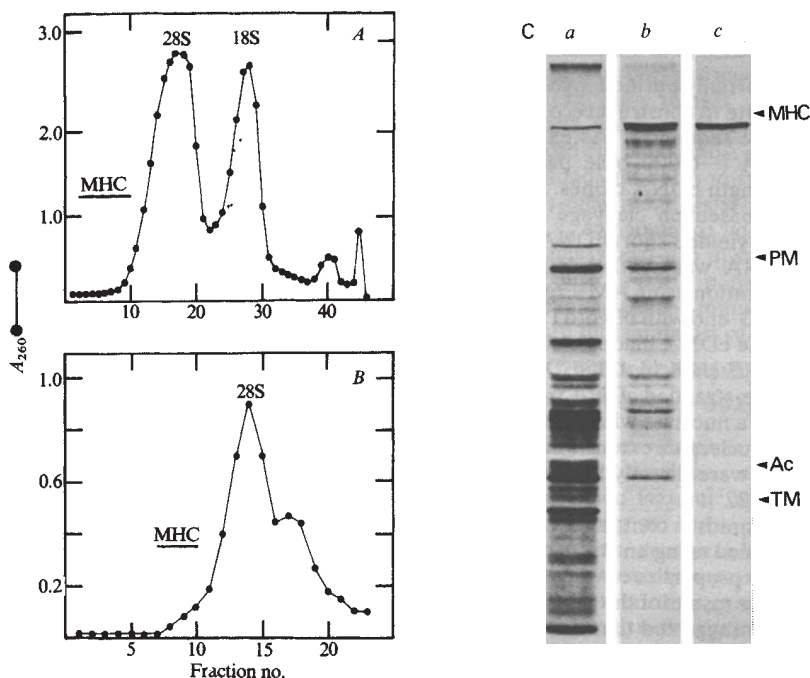
The myosin heavy chains of MWs 205,000 and 200,000 seem to correspond to the previously identified pharyngeal and residual body wall myosin heavy chains, respectively²⁻⁴. We also detect a previously uncharacterized minor component of MW 210,000 in E675 and E190 animals. We assume that N2 animals also synthesize this product but that its presence is obscured by the *unc-54* myosin heavy chain. This component is not synthesized *in vitro* in either the wheat-germ or rabbit reticulocyte cell-free systems and has not been characterized further.

Purification of the *unc-54* mRNA

The preceding experiments demonstrate that cell-free translation of nematode mRNAs in a heterologous protein-synthesizing system provides a reliable assay for *unc-54* mRNA activity. We applied this assay to monitor the purification of the myosin heavy-chain mRNAs. Because of their large size a considerable purification of these mRNAs can be achieved by successive sedimentation through neutral and denaturing¹⁵ sucrose gradients. Figure 2 shows a typical purification of *unc-54* mRNA.

Although a substantial purification of the *unc-54* myosin heavy-chain mRNA has been achieved, this fraction still contains the mRNAs coding for minor myosin heavy chains as well as small amounts of mRNAs coding for unidentified proteins of lower molecular weight (Fig. 2C), and ribosomal RNA sequences which account for much of the nucleic acid mass. These stable RNA contaminants can, in principle, be removed by affinity chromatography of the *unc-54* mRNA on oligo(dT)-cellulose¹⁶ or poly(U)-Sephacrose¹⁷. However, we have found that nematode myosin heavy-chain mRNAs, like other large mRNAs¹⁸, are not retained efficiently on these columns, apparently due to steric hindrance rather than to the absence of a 3'-poly(A) tract.

Fig. 2 Purification of the *unc-54* mRNA. RNA (30 mg) from nematode larvae was dissolved in 10 ml of 10 mM Tris-HCl pH 7.5, 1 mM EDTA and 10% dimethyl sulfoxide, heated at 65 °C for 10 min and loaded on a 10–40% sucrose gradient in 100 mM NaCl, 10 mM Tris-HCl pH 7.5, 1 mM EDTA and 0.1% SDS generated in an MSE type XIV zonal rotor. After centrifugation at 40,000 r.p.m. for 15 h the gradient was unloaded and fractions containing myosin heavy-chain mRNA activity were identified by cell-free translation. These fractions were pooled, ethanol precipitated and redissolved in water at a concentration of 10 mg ml⁻¹. This was adjusted to 20 mM sodium borate pH 8.0 and 50 mM methylmercury hydroxide (Alfa)¹⁵. A 300- μ l aliquot was loaded on a 13-ml 5–20% sucrose gradient in 100 mM NaCl, 10 mM Tris-HCl pH 7.5, 1 mM EDTA and 0.1% SDS. The gradient was centrifuged in the Beckman SW40 at 40,000 r.p.m. for 5 h. **A**, optical density profile of RNA fractionated in the zonal rotor. **B**, optical density profile of RNA refractionated after treatment with methylmercury hydroxide. **C**, assay of myosin heavy-chain mRNA purification by cell-free protein synthesis. **a**, Proteins synthesized in response to total larval RNA; **b**, proteins synthesized in response to the myosin mRNA purified by zonal gradient centrifugation, as shown in **A**; **c**, proteins synthesized in response to myosin mRNA repurified by the denaturing sucrose gradient, shown in **B**.



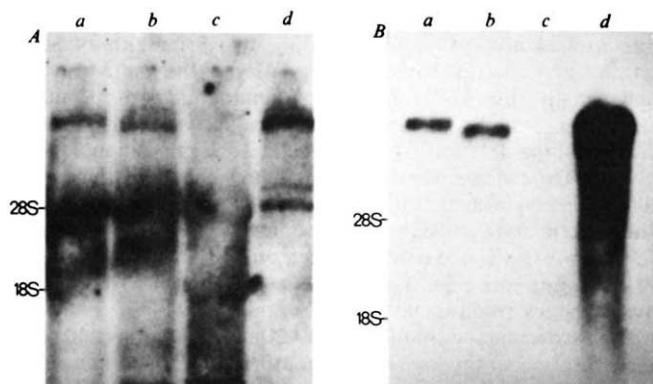


Fig. 3 Identification of plasmids containing sequences complementary to the *unc-54* mRNA. cDNA was synthesized¹⁹ in a 150- μ l reaction containing 50 mM KCl, 50 mM Tris-HCl pH 8.3, 10 mM Mg-acetate, 2 mM DTT, 1 mM each of dGTP, dCTP, dTTP, 200 μ M dATP and 50 μ Ci [α -³²P]dATP (Radiochemical Centre), 30 μ g ml⁻¹ oligo(dT), 5 μ g enriched *unc-54* mRNA and 40 units of AMV reverse transcriptase (gift of J. Beard). RNA was hydrolysed by incubation in 0.1 M NaOH and 1 mM EDTA³⁹ at 70 °C for 20 min, desalted⁴⁰ and the cDNA was made double stranded in a 100- μ l reaction containing 100 mM KCl, 50 mM HEPES adjusted to pH 6.9 with KOH, 5 mM Mg-acetate, 1 mM DTT, 500 μ M each dGTP, dCTP, dTTP, dATP and 50 μ g ml⁻¹ of DNA polymerase²⁰. Single-stranded cDNA was digested with 8 units of S₁ nuclease¹⁸ at 37 °C for 45 min in a 50- μ l reaction mixture containing 200 mM NaCl, 50 mM Na-acetate pH 4.6 and 3 mM ZnCl₂. Fractions containing high molecular weight double-stranded cDNA eluting from a Sepharose 4B column were pooled and digested with 5 units *Mbo*I²¹ at 37 °C for 30 min in 6 mM NaCl, 6 mM Tris-HCl pH 7.5, 6 mM Mg-acetate and 6 mM 2-mercaptoethanol. The *Mbo*I-digested DNA was ligated to *Bam*-digested pBR322²² (previously treated with alkaline phosphatase⁴¹) in a 50- μ l reaction containing 60 mM Tris-HCl pH 7.6, 8 mM Mg-acetate, 7 mM 2-mercaptoethanol, 0.25 μ g vector DNA and 20 μ g ml⁻¹ T4 ligase. After incubation at 10 °C for 1 h, the reaction was diluted to 100 μ l and mixed with 200 μ l CaCl₂-treated cells⁴². Transformed cells harbouring plasmids containing inserts were selected as ampicillin-resistant, tetracycline-sensitive colonies. Plasmid DNA was prepared from colonies which hybridized to cDNA⁴³ by the caesium chloride-ethidium bromide centrifugation procedure⁴⁴. These were labelled with [α -³²P]dATP by nick translation (see Fig. 6 legend) and tested for the presence of *unc-54* sequences by hybridization to filters containing fractionated nematode RNA. RNA from N2, E675 and E190 animals and the partially purified *unc-54* mRNA were separated in a 1.5% agarose gel in TBE buffer (90 mM Tris (base), 90 mM boric acid, 2.5 mM EDTA pH 8.3) containing 0.1% SDS. The RNA was transferred to diazotized paper²³ and hybridized to labelled probes. **A**, ³²P-cDNA prepared from enriched *unc-54* mRNA probe. **B**, nick-translated pMbo7 DNA probe. In each panel: **a**, N2 RNA; **b**, E675 RNA; **c**, E190 RNA; **d**, partially purified *unc-54* mRNA prepared by sucrose gradient centrifugation.

Detection of *unc-54* mRNA with cDNA clones

The partially purified myosin heavy-chain mRNA was used as a template to construct recombinant plasmids containing *unc-54*-specific sequences. As expected, the large size of the *unc-54* mRNA (~6,000 base pairs) prevented us from synthesizing full-length cDNA clones. We therefore devised a cloning strategy, based on cleavage of cDNA with restriction enzymes, which yielded short cDNA probes of the desired specificity.

cDNA was synthesized from the enriched *unc-54* mRNA preparation using AMV reverse transcriptase selectively primed at the 3' end with oligo(dT)¹⁹. The template RNA was destroyed and the cDNA made double stranded in a self-primed reaction using *Escherichia coli* DNA polymerase A²⁰. Fragments of double-stranded cDNA were then generated²¹ by cleavage with *Mbo*I, a nuclease which cleaves at the sequence GATC leaving a tetranucleotide extension. These short fragments (100–500 base pairs) were directly ligated into the *Bam*HI site of the plasmid pBR322²².

Plasmids containing *unc-54*-specific sequences were identified using an RNA blotting and hybridization assay based on the properties of wild-type and mutant *unc-54* mRNAs (Fig. 3). The results of the cell-free translation experiments described above suggested that the *unc-54* mRNA should be reduced in molecular weight by ~300 nucleotides in E675 animals and in low abundance in E190 animals. These differences should be

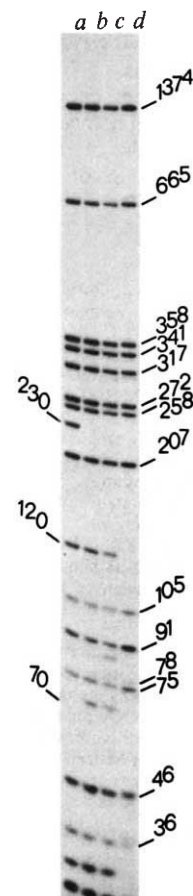
detectable by hybridization of cloned *unc-54* sequences to RNA prepared from these strains, which has been size fractionated by gel electrophoresis. Because the genetic evidence unambiguously assigns the *e675* and *e190* mutations to a single locus, these strain differences should only be detected when *unc-54* sequences are used as hybridization probes.

A control experiment using ³²P-labelled cDNA prepared from the enriched *unc-54* mRNA fraction demonstrates the specificity of this assay. Total RNA from wild-type N2, E675 and E190 animals and the *unc-54* mRNA fraction were fractionated on 1.0% agarose gels and transferred to activated paper²³. Hybridization of cDNA to the partially purified *unc-54* mRNA fraction detects a number of RNA molecules (Fig. 3A). The major component of this fraction shows the expected properties of *unc-54* mRNA. This component is detected in the total RNA fraction from wild-type animals probed with ³²P-labelled *unc-54* cDNA and is reduced in size in E675; however, a similar RNA species cannot be detected in E190 RNA. This experiment provides unambiguous genetic evidence that *unc-54* mRNA has been detected with this probe.

Figure 3B shows the hybridization pattern obtained when a plasmid containing *unc-54* sequences is used as a probe in the assay (pMbo7). A single RNA species is detected in the N2 RNA fraction which shows the expected reduction in size in E675 and is absent from E190. No E190 sequences were detected with this probe, indicating either that the E190 mRNA is present below the level of detection in this experiment or that the *unc-54*-specific sequence contained in the pMbo7 probe is absent from the altered mRNA.

The structures of three plasmids containing *unc-54* cDNA sequences are shown in Fig. 4. Plasmid DNA was digested with *Sau*3A²⁴ (an isoschizomer of *Mbo*I) and the restriction fragments separated by polyacrylamide gel electrophoresis. In addition to the restriction fragments of the vector, all the recombinants carry a fragment of 120 base pairs and pMbo7 and pMbo8 also carry a fragment of 70 base pairs. This suggests that these two restriction fragments are contiguous in the *unc-54*

Fig. 4 Structure of recombinant plasmids containing *unc-54* cDNA sequences. Covalently closed circular DNA was isolated by the caesium chloride-ethidium bromide centrifugation procedure⁴⁴. Plasmid DNA (1 μ g) was digested with 2 units *Sau*3A²⁴ for 60 min at 67 °C in 10 mM Tris-HCl pH 7.4, 50 mM NaCl, 10 mM MgCl₂ and 10 mM DDT and was end labelled by incubation in the presence of 1 μ Ci [α -³²P]dATP, 500 μ M each of nonradioactive dGTP, dCTP, dTTP and 1 unit of AMV reverse transcriptase. The labelled fragments were fractionated by electrophoresis through a thin (0.03 $\times$ 20 $\times$ 40 cm) 5% polyacrylamide gel⁴⁵ without urea in TBE buffer and detected by autoradiography of the dried gel. The sizes of the *Sau*3A restriction fragments of pBR322, based on nucleic acid sequence information⁴⁶, are indicated on the right side of the figure. The sizes of inserted *Sau*3A fragments are indicated on the left side. **a**, pMbo3; **b**, pMbo7; **c**, pMbo8; **d**, pBR322.



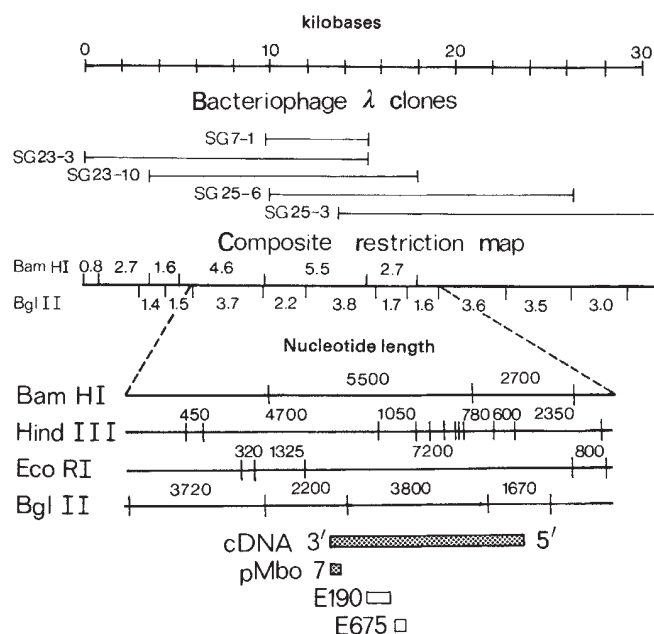


Fig. 5 Restriction endonuclease maps of clones containing the *unc-54* myosin heavy chain and flanking sequences. Recombinant phages were prepared as described using bacteriophage λ 1059 as a vector³⁰. Representative clones are shown in the top panel. The maps are drawn without the phage sequences. SG 7-1 is a *Bam*HI clone containing the *Bam* 5.5-kilobase fragment and a second restriction fragmentation of 12-kilobases (not shown). SG 23-3 and SG 23-10 are clones obtained from *Bam*HI partial digestion of nematode DNA. They share the 5.5- and 4.6-kilobase *Bam*HI fragments but differ in their flanking sequences. SG 25-6 and SG 25-3 were obtained from clones prepared by *Sau*3A digestion of nematode DNA. They both have one end point within the *Bam* 5.5-kilobase fragment and extend leftward. A composite *Bam*HI-*Bgl*II restriction map of the 30-kilobase region defined by these overlapping clones is drawn below the phage maps. The fragment sizes are given in kilobases. A more detailed restriction map of part of this region is also drawn. The length of the fragments in nucleotides is indicated. The small unnumbered *Hind*III fragments have sizes of 294, 368, 357, 117 and 111 nucleotides, reading from left to right. The sites of hybridization of pMbo7 and cDNA to *unc-54* mRNA are indicated by the cross-hatched bars. Note that the end point of the detected cDNA hybridization does not precisely define the 5' end of the *unc-54* mRNA sequence. The open bars indicate the positions of the *e*675 and *e*190 deletions. These positions were assigned on the basis of Southern transfer hybridization experiments of the type shown in Fig. 6.

mRNA sequence and were cloned together in pMbo7 and pMbo8 by incomplete digestion of double-stranded cDNA. The plasmid pMbo3 also carried an additional unique fragment of 230 base pairs which does not hybridize to *unc-54* mRNA, and was probably cloned by a multiple insertion event.

*e*190 is a small deletion

The cDNA plasmids can be used to identify restriction fragments of nematode genomic DNA which contain *unc-54* sequences. *Bam*HI-digested DNA isolated from N2, E675 and E190 animals was hybridized to nick-translated²⁵ pMbo7 (Fig. 6A) using the method of Southern²⁶. A single labelled restriction fragment of 5.5 kilobases is detected in wild-type DNA. It is reduced in size in E675 by approximately 300 base pairs, consistent with the size of the internal deletion in the E675 myosin heavy chain. Surprisingly, this fragment shows a reduction in size of ~600 base pairs in E190, demonstrating that the *e*190 mutation is also a small deletion. As the sequence in the pMbo7 probe is present in the genomic DNA but is undetectable in fractions expected to contain *e*190 mRNA, this result suggests that the *e*190 mutation produces a more complicated alteration of the *unc-54* transcription unit than might be expected to result from a simple deletion of part of the coding sequence, as in E675.

To map the *unc-54* gene more precisely, we turned our attention to molecular cloning of genomic DNA sequences.

Molecular cloning of the *unc-54* gene

Eukaryotic genes have been isolated from collections of randomly cloned DNA fragments²⁷⁻²⁹. We have recently described a novel bacteriophage λ cloning vector, λ 1059, which has been used to construct a collection of recombinants covering the *C. elegans* genome³⁰. High-molecular-weight N2 DNA was digested with *Bam*HI or *Sau*3A in conditions which yielded fragments of 15-20 kilobases. These were purified by agarose gel electrophoresis and ligated to the bacteriophage arms. Recombinants harbouring inserts were then selected by growth on a strain which restricted the growth of the vector.

Twenty-two recombinants which hybridized to cDNA and cDNA plasmid probes³¹ were isolated and the restriction maps of each determined. These phage were found to contain a series of overlapping restriction fragments which could be ordered to generate a map extending for about 30 kilobases of the nematode genome. The composite restriction map of *Bam*HI and *Bgl*II fragments and the end points of four of these phages are shown in Fig. 5.

Orientation of the gene

The short cDNA plasmid pMbo7 hybridizes to the site within the *Bam* 5.5-kilobase fragment indicated in Fig. 5 (data not shown). The orientation of the *unc-54* gene can be defined with respect to this site by comparing the patterns of hybridization of *unc-54* cDNA and pMbo7. Restriction fragments to the left side of the site of pMbo7 hybridization (as the map is drawn) do not hybridize to cDNA while those to the right will hybridize efficiently (Fig. 5). Since cDNA synthesis is primed by oligo(dT) at the 3'-poly(A)-terminus of the *unc-54* mRNA this suggests that pMbo7 contains a sequence at or near the 3' end of the transcript. The fact that because of the very large size of the *unc-54* mRNA very few cDNA transcripts primed with oligo(dT) were of full length introduces some ambiguity in mapping the 5' end of the *unc-54* coding sequence, and the map position indicated in Fig. 5 is accurate to only 300 base pairs. Preliminary evidence using mRNA as a probe in Berk-Sharp³²

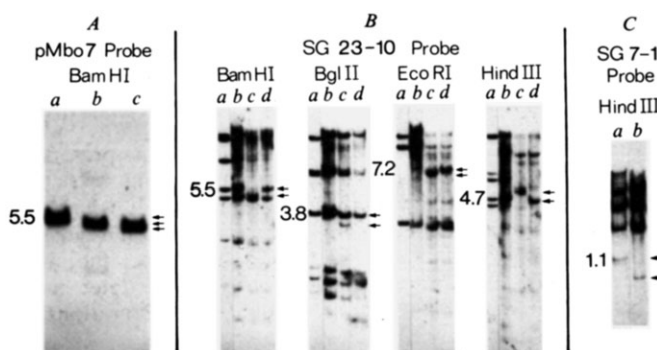


Fig. 6 Identification of *unc-54* DNA sequences in restriction fragments of nematode genome DNA. DNA was isolated from nematodes essentially as described previously³³. DNA (10 μ g) was digested with various restriction enzymes in a 50- μ l reaction containing 50 mM KCl, 10 mM Tris-HCl pH 7.5 and 6 mM Mg acetate and electrophoresed in agarose gel in TAE buffer (40 mM Tris-acetate pH 7.3, 20 mM Na-acetate, 2 mM EDTA) and transferred to nitrocellulose²⁶. Bacteriophage were grown as liquid lysates on Q358 as described previously³⁰. Aliquots (10 ng) of phage DNA were digested with restriction enzymes and used as molecular weight markers (a). Probes for Southern transfer experiments were prepared by end labelling of restriction fragments with AMV polymerase as described in Fig. 4 legend. The probe obtained from pMbo7 was a *Hind*III-*Sal* fragment which was purified by gel electrophoresis before hybridization to DNA immobilized on nitrocellulose. The bacteriophage probes were end-labelled *Sau*3A digests of the phage DNA. A, hybridization of pMbo7 to *Bam*HI-cleaved DNA from N2 (a), E675 (b) and E190 (c) fractionated on a 0.8% agarose gel. B, hybridization of SG23-10 DNA to *Bam*HI, *Bgl*II, *Eco*RI, *Hind*III cleaved DNA from N2 (b), E190 (c) and E1201 (d), fractionated on a 1% agarose gel. C, hybridization of SG 7-1 DNA to *Hind*III-cleaved DNA from N2 (a) and E675 (b) fractionated on a 1.5% agarose gel. Numbers indicate the size in kilobases of restriction fragments that are affected by the E190 and E675 deletions. These fragments are similarly identified in the restriction map in Fig. 5. Arrows indicate the positions of the altered restriction fragment in the wild-type and mutant strains.

hybridization experiments also places the 5' end of the messenger to the indicated position within the *Bam* 2.7-kilobase fragment.

Mapping *e675* and *e190*

We confirmed the results of this mapping experiment by more accurate mapping of the *e675* and *e190* deletions. As our chemical cleavage studies had shown that the terminal 300–350 amino acids of the *unc-54* myosin heavy chain are present in E675⁸, this mutation should be located approximately 1 kilobase from the end of the *unc-54* mRNA. A similar map position should be observed in the genome, assuming that there are few intervening sequences in this region. Cell-free translation data can be used to predict the location of the *e190* deletion. As described above, RNA isolated from E190 programmes the synthesis of a polypeptide of MW 170,000 which is immunoprecipitated by anti-myosin antiserum. A polypeptide of this size could be produced by deletion of the terminal 350 amino acid residues of the *unc-54* myosin heavy chain. In this case, *e190* should map near *e675* at ~1,000 base pairs from the end of the transcribed sequence.

The Southern transfer procedure was used to map the positions of these two mutants. DNA from wild-type, E190, E675 and E1201 animals (a recessive *unc-54* mutant) was digested with a variety of restriction enzymes and probed with radioactive SG 23-10 or SG 7-1 DNA (Fig. 6). The *e190* deletion produces several alterations in the cleavage patterns (Fig. 6B, panel 1). In particular, this mutation results in the fusion of the 4.8- and 1.1-kilobase *Hind*III fragments found in the wild-type pattern, localizing the *e190* deletion to the region indicated in Fig. 5. Similarly, the *e675* deletion has been localized within the 1.1-kilobase *Hind*III fragment (Fig. 6C). The two mutations thus seem to map close to one another and may, in fact, overlap. This provides evidence for our interpretation of the E190 mRNA translation pattern and confirms that the *unc-54* gene is oriented as shown in Fig. 5. No alteration of the restriction map of E1201 was detected in these experiments, suggesting that this recessive null mutation may be a single base pair change.

These Southern transfer experiments also confirm the restriction map derived from an analysis of the bacteriophage λ clones. No rearrangements seem to have occurred during the cloning procedure because each of the restriction fragments present in the nematode- λ recombinants are also detected in the genomic DNA.

In addition to the strongly hybridizing fragments present in the cloned probes, a number of weakly hybridizing restriction fragments were detected in these experiments. It will be of interest to determine whether any of these restriction fragments show homology to *unc-54* sequences, and whether the other myosin heavy chains present in *C. elegans* can be detected by hybridization to *unc-54*-specific sequences in conditions of relaxed stringency.

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1. Brenner, S. *Genetics* **77**, 71–94 (1974).
2. Epstein, H. F., Waterston, R. H. & Brenner, S. *J. molec. Biol.* **90**, 291–300 (1974).
3. MacKenzie, J. M., Schachat, F. & Epstein, H. F. *Cell* **15**, 413–419 (1978).
4. MacLeod, A. R., Waterston, R. H., Fishpool, R. M. & Brenner, S. *J. molec. Biol.* **114**, 133–140 (1977).
5. Schachat, F., Harris, H. E. & Epstein, H. F. *Cell* **10**, 721–728 (1977).
6. Riddle, D. L. & Brenner, S. *Genetics* **89**, 299–314 (1978).
7. Waterston, R. H. & Brenner, S. *Nature* **275**, 715–719 (1978).
8. MacLeod, A. R., Waterston, R. H. & Brenner, S. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5336–5340 (1977).
9. Cox, R. A. *Meth. Enzym.* **12B**, 120–129 (1968).
10. Roberts, B. E. & Paterson, B. M. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2330–2334 (1973).
11. Pelham, H. R. B. & Jackson, R. J. *Eur. J. Biochem.* **67**, 247–256 (1976).
12. MacLeod, A. R., Karn, J., Waterston, R. H. & Brenner, S. in *Nonsense Mutations and tRNA Suppressors* (eds Celis, J. E. & Smith, J. D.) 301–311 (Academic, New York, 1979).
13. Jacobson, G. R., Schaffer, M. H., Stark, G. R. & Vanaman, T. C. *J. biol. Chem.* **248**, 6583–6591 (1973).
14. Degani, Y. & Patchornik, A. *Biochemistry* **13**, 1–11 (1974).
15. Bailey, J. M. & Davidson, N. *Analyt. Biochem.* **70**, 75–85 (1976).
16. Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408–1412 (1972).
17. Adesnick, M., Saldith, M., Thomas, W. & Darnell, J. E. *J. molec. Biol.* **71**, 21–30 (1972).
18. Vogt, V. M. *Eur. J. Biochem.* **33**, 192–200 (1973).
19. Buell, G. N., Wickens, M. P., Payvar, F. & Schimke, R. T. *J. biol. Chem.* **253**, 2471–2482 (1978).
20. Wickens, M. P., Buell, G. N. & Schimke, R. T. *J. biol. Chem.* **253**, 2483–2495 (1978).
21. Gelin, R. E., Myers, P. A. & Roberts, R. J. *J. molec. Biol.* **114**, 169–180.
22. Bolivar, F. *et al. Gene* **2**, 95–113 (1977).

The *unc-54* gene

The *unc-54* myosin heavy-chain gene of *C. elegans* provides an attractive model system for studying molecular genetics in a multicellular higher organism. Numerous mutants in the gene have been isolated, and more can be readily obtained.

Here we have described a series of complementary biochemical assays which may be used to define the molecular phenotypes of these mutations. Myosin synthesis can be studied both *in vivo* and *in vitro* by cell-free translation of the *unc-54* mRNA and immunoprecipitation of the myosin heavy chains. Messenger RNA can be detected by hybridization to cloned probes and deletions can be mapped by Southern hybridization transfer experiments.

In developing these assays we relied on the properties of a mutant, E675, which was known to contain an internal deletion of 100 amino acids near the carboxy terminus of the *unc-54* myosin heavy chain⁸. Thus, *unc-54*-specific translation products, mRNA molecules and restriction fragments of the nematode genome could be identified as molecules which showed altered length in E675. This assay allowed us to identify sequences from the active gene in cloning experiments and to distinguish the products of the *unc-54* gene from those of the other myosin heavy-chain genes present in this organism.

We have also applied these assays to E190¹ and E1201⁴. E190 was the first mutant isolated in *unc-54*¹ and was long thought to be a point mutation. However, Southern transfer experiments revealed that *e190* is, in fact, a small deletion near the 3' end of the *unc-54* coding sequence. Unlike E675, E190 does not produce a stable protein product and appears deficient in *unc-54* mRNA. However, immunoprecipitation of the myosin heavy chains synthesized *in vitro* by E190 RNA reveals the presence of a minor polypeptide specific to E190, which may be a truncated translation product of the *unc-54* (*e190*) mRNA. The size and position of the deletion found in E190 DNA are consistent with this interpretation. In contrast to E190, E1201 shows no alteration of the genomic restriction map, suggesting that this recessive null mutation may be the result of a single base pair change. However, in other respects the phenotypes of E1201 and E190 are similar. Both mutants are deficient in *unc-54* mRNA synthesis and both code for immunoprecipitable peptides which may be detected by cell-free translation¹².

Further analysis of the structure of the wild-type *unc-54* gene and of these and other mutants should contribute to our knowledge of gene expression in eukaryotes as well as to the molecular biology of muscle.

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23. Alwine, J. C., Kemp, P. J. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5350–5354 (1977).
24. Liu, A. C. P., McBride, B. C., Vovis, G. F. & Smith, M. *Nucleic Acids Res.* **6**, 1–15 (1979).
25. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237–251 (1977).
26. Southern, E. M. *J. molec. Biol.* **8**, 503–517 (1975).
27. Wensink, P. C., Finnegan, D. J., Donelson, J. E. & Hogness, D. *Cell* **3**, 315–325 (1974).
28. Kedes, L. H., Chang, A. C. Y., Houseman, D. & Cohen, S. N. *Nature* **255**, 533–537 (1975).
29. Maniatis, T. *et al. Cell* **15**, 687–701 (1978).
30. Karn, J., Brenner, S., Barnett, L. & Cesareni, G. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5172–5176 (1980).
31. Benton, W. P. & Davis, R. W. *Science* **196**, 180–182 (1977).
32. Berk, A. J. & Sharp, P. A. *Cell* **12**, 721–732 (1977).
33. Sulston, J. E. & Brenner, S. *Genetics* **77**, 95–104 (1974).
34. Kessler, S. W. *Immunology* **115**, 1617–1624 (1975).
35. Degani, Y. & Patchornik, A. *J. org. Chem.* **36**, 2727–2728 (1971).
36. Laemmli, U. K. *Nature* **227**, 680–685 (1970).
37. Bonner, W. M. & Laskey, R. A. *Eur. J. Biochem.* **46**, 83–88 (1974).
38. Laskey, R. A. & Mills, A. D. *Eur. J. Biochem.* **56**, 335–341 (1975).
39. Seeburg, P. H., Shine, J., Martial, J. A., Baxter, J. D. & Goodman, H. M. *Nature* **270**, 486–494 (1977).
40. Brownlee, G. G. & Cartwright, E. M. *J. molec. Biol.* **114**, 93–117 (1977).
41. Deeley, R. G. *et al. J. biol. Chem.* **252**, 8310–8319 (1977).
42. Cohen, S. N., Chang, A. C. Y., Boyer, H. W. & Helling, R. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3240–3244 (1973).
43. Grunstein, M. & Hogness, D. S. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3961–3965 (1975).
44. Clewell, D. B. *J. Bact.* **110**, 667–676 (1972).
45. Sanger, F. & Coulson, A. R. *FEBS Lett.* **87**, 107–110 (1978).
46. Sutcliffe, J. G. *Nucleic Acids Res.* **5**, 2721–2728.

LETTERS TO NATURE

Why is the Universe open?

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One of the most fundamental properties of the Universe is its density, or more usefully the ratio $\Omega = \rho/\rho_c$ of the density to the critical value required for closure. The feeling among cosmologists is that the Universe is open, with Ω perhaps in the range 0.1–0.5 (see ref. 1). In general, Ω is thought of as a parameter to be deduced from observation, but some reasons for it having a particular value have been suggested; there is a certain aesthetic appeal in having $\Omega = 1$ (ref. 2), and the 'anthropic principle'³ suggests that Ω should not be too far from unity if the Universe is to evolve in such a way as to allow the development of stars, and thence ourselves. Here speculations are made concerning the transition from quantum to classical gravitational behaviour near the initial cosmic singularity, and a physical process is proposed whose action leads naturally to a Universe which is open.

In a Universe which is, on average, represented by an $\Omega = 1$ Friedmann model, an inhomogeneity in the form of a density enhancement is a region of local positive curvature. There exists a general argument⁴ to say that if the density enhancement is too great, the curvature will be large enough for the region to close up on itself and be completely disconnected from the $\Omega = 1$ Universe. In classical general relativity, such disjoint regions never have been, and never will be in contact, but I suggest here that their mutual independence may be removed by quantum gravitational effects.

Quantum effects dictate that the precise definition of classical points, surfaces, and so on becomes impossible on small scales⁵. One must therefore imagine that the picture of the classical Universe as an ordered set of well-defined spacelike hypersurfaces breaks down near the Planck time, $t_p \approx 10^{-43}$ s, as quantum fluctuations of the metric disrupt and mix up the classical surfaces⁶. The proposal made here is that the regions of density enhancement mentioned above may become physically separated, in a dynamical sense, from the rest of the Universe during the transition from quantum to classical eras: in the classical era the spacetime manifold is well-defined, and the regions are disjoint, but in the quantum era, everything is smeared together.

One can usefully envisage this process occurring in 'superspace', each point of which represents a particular spacetime geometry⁵. Classically, any cosmological model follows a trajectory of least action through superspace, but using a path integral approach to quantum gravity⁷, one must consider all routes, including non-classical ones, between one geometrical configuration and the next. The present conjecture then amounts to a supposition that the Universe, as it emerges from the region of superspace where quantum gravity dominates, can reach configurations which are topologically inequivalent to Friedmann models: that is, they comprise several unconnected regions. There seems to be no very strong arguments at present for or against this hypothesis, but the consequences would be interesting.

A simple calculation will show how the density of the Universe may be determined by its initial conditions. If an inhomogeneity has, at t_p , mass m and density contrast $\delta = (\rho' - \rho)/\rho$, then the criterion that it should form a separate region⁶ is

$$\delta > \delta_c = \alpha(m/m_0)^{-2/3} \quad (1)$$

where m_0 is the horizon mass at t_p , and α is a constant of the

order of one. Now suppose, following Carr⁸, that the spectrum of density fluctuations is gaussian, with width $\delta_{rms} = \epsilon(m/m_0)^{-n}$. If $n < 2/3$, inhomogeneity is greater on larger scales (in comparison with δ_c), and this case must be excluded as it does not correspond, for large m , to perturbations on a smooth background. If $n = 2/3$, inequality (1) is independent of m ; this case will be mentioned later. For the moment, we assume $n > 2/3$, in which case fluctuations with $m = m_0$ are most important, and those with $m > m_0$ can be neglected.

The fraction of fluctuations with $\delta > \delta_c$ is roughly⁸ $P = \exp(-x^2)/x$, where $x = \alpha/\sqrt{2\epsilon}$. P can be taken to be the fraction of matter which is 'lost' in the quantum to classical transition. (If one supposed the fluctuations not to be spherically symmetric, and that P were the probability of the curvature closing on itself in one space direction, then it could be argued that P^3 would be the fraction of matter lost. This in fact makes little difference).

If Ω_0 is the cosmic density ratio today, and Ω_p its value at t_p , then it is easy to show that

$$1 - \Omega_0 = (1 - \Omega_p)(t_{eq}/t_p)(t_0/t_{eq})^{2/3} \quad (2)$$

where the Universe is radiation dominated from $t \approx 10^{-43}$ s to $t_{eq} \approx 10^{12}$ s, and matter dominated from t_{eq} to the present, $t_0 \approx 10^{18}$ s. If the Universe is noticeably open now ($\Omega_0 < 1$), then $\Omega_p \approx 1 - 10^{-60}$. We now have a possible explanation for the tiny, but non-zero, departure from the $\Omega = 1$ 'ideal': a small fraction of the cosmic density was lost at the beginning. Taking P (or P^3) to be 10^{-60} requires $\alpha/\epsilon = 15$ (or 10), which seems plausible; $\epsilon = 1$ represents a chaotic initial state, with lower values signifying a more uniform Universe, and it might be expected that the simple analysis leading to equation (1) underestimates the difficulty with which separation of regions occurs, so that α might be rather larger than one.

In the particular case $n = 2/3$, the probability of regions becoming separate is independent of their size; thus one might imagine a whole 'family' of Universes emerging from the quantum regime. However, there is a problem in that the observed large-scale structure of the Universe suggests that the magnitude of $n = 2/3$ fluctuations is perhaps $\epsilon = 10^{-4}$ (ref. 9), so that the probability of there being any disjoint regions would be very small ($P \approx \exp(-10^8)$).

It would be a mistake to take this calculation seriously in any quantitative way, and although the numbers fit together quite well, we cannot claim to have predicted the density of the Universe because the path of cosmological evolution is extremely sensitive to the value of Ω_p . In fact, if α/ϵ has a value of 1 rather than 10, a large fraction of the density will be lost at t_p , and the Universe will expand and recontract far too quickly to allow stars and galaxies to develop. Low values of α/ϵ clearly do not lead to Universes like our own, and although a reason has been suggested why $\Omega < 1$, there still seems to be some sort of anthropic coincidence in the fact that Ω_0 is only slightly less than 1 after 10^{10} yr. Another puzzle lies in finding a sounder physical basis for the assumption that $\Omega = 1$ is a 'preferred' state; the idea that such a Universe has an exact balance between kinetic and gravitational energy² is interesting, but, at present, no more than suggestive.

Recently, Guth¹⁰ has looked at a possible explanation for some of the gross features of the Universe, but the mechanism he uses (phase transitions in Grand Unified Theories) is completely different from the one discussed here; therefore no direct comparison of the two approaches seems possible. Nevertheless, it is a new and intriguing idea that the density of the Universe may be, as the cosmic baryon number has recently been shown to be, a predictable quantity.

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- Gott, J. R. III, Gunn, J. E., Schramm, D. N. & Tinsley, B. M. *Astrophys. J.* **194**, 543–554 (1974).
- Dicke, R. H. & Peebles, P. J. E. in *General Relativity: an Einstein Centenary Survey* (eds Hawking S. W. & Israel, W.) 504–517 (Cambridge University Press, 1979).
- Carter, B. in *Confrontation of Cosmological Theories with Observational Data* (ed. Longair, M. S.) 291–298 (Reidel, Dordrecht, 1974).
- Carr, B. J. & Hawking, S. W. *Mon. Not. R. astr. Soc.* **168**, 399–415 (1974).
- Misner, C. W., Thorne, K. S. & Wheeler, J. A. *Gravitation* (Freeman, San Francisco, 1973).
- Harrison E. R. *Phys. Rev. D* **1**, 2726–2730 (1970).
- Hawking, S. W. in *General Relativity: an Einstein Centenary Survey* (eds Hawking, S. W. & Israel, W.) 746–789 (Cambridge University Press, 1979).
- Carr, B. J. *Astrophys. J.* **201**, 1–19 (1975).
- Gott, J. R. III *A. Rev. Astr. Astrophys.* **15**, 235–266 (1977).
- Guth, A. H. *Phys. Rev. D* **23**, 347–356 (1981).

IR variability of SS433

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SS433 shows X-ray, optical and radio variability and evidence of material moving at one-quarter the speed of light¹. We report here the presence of the 165.5-day spectroscopic period in our extensive *JHKL* (1.2–3.4 μm) photometry. This indicates that the jets, rather than a highly reddened O star, are a major source of the IR radiation. We also show that the (*J*–*H*) colour index becomes redder as SS433 brightens but find no evidence for the 11.8-day period reported by Giles *et al.*².

Seventy-two *JHK* observations of SS433 have been made at the South African Astronomical Observatory with the 1.88- and 0.75-m telescopes. The observations span three observing seasons and include a run in 1980, when the star was observed on 33 out of 55 consecutive nights. The data and standard errors are listed in Table 1. The errors contain an 'internal' standard error of observation and an 'external' error which is fixed at $\Delta J = \pm 0.06$, $\Delta H = \pm 0.05$, $\Delta K = \pm 0.04$ and $\Delta L = \pm 0.04$ for the 0.75-m telescope and at ± 0.03 for all filters on the 1.88-m telescope. We list *L* magnitudes obtained only with the 1.88-m telescope although other less accurate ones, made with the 0.75-m telescope, are available. The observations have all been reduced to the system of Glass³ standards. On six occasions in 1979 Giles *et al.* and ourselves observed SS433 within 6 h of each other. For these nights our observations are systematically brighter by 0.13 in *J*, and 0.06 mag in *H* and *K*. The simultaneous observations cover a range of *K* magnitudes from 8.3 to 7.2 and so independently confirm the reality of the rapid change in brightness which we have observed. We have not applied any systematic corrections to the data from Giles *et al.* because we cannot assume that such corrections are universally applicable to their rather heterogeneous observations.

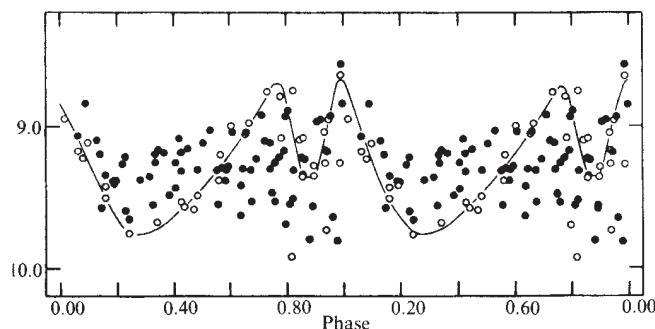


Fig. 1 The *J* magnitude as a function of phase for an 11.8-day period. ●, Data in Table 1. ○, Data given by Giles *et al.* The curve is their proposed light curve. Phase 0.0 is on JD 2443718.9.

A sequential plot of the data shows that the brightness in all colours fluctuates by tenths of a magnitude on a time scale of days while occasional brightening and fadings of 0.7 mag occur on the time scale between 4 and 5 days. Clearly any underlying periodicities will probably be obscured by this 'flickering'. Period-finding programmes must therefore be used with great care as the occurrence of a few strong brightenings, coupled with the very patchy distribution of data, can cause spurious periodicities.

Table 1 *JHKL* observations of SS433

JD	<i>J</i> ± ΔJ	<i>H</i> ± ΔH	<i>K</i> ± ΔK	<i>L</i> * ± ΔL *
3729.40	9.56 0.07	8.94 0.05	8.19 0.04	
3730.43	9.81 0.07	9.19 0.06	8.44 0.07	
3732.43	9.58 0.06	9.03 0.07	8.28 0.05	
3733.43	9.60 0.06	9.01 0.05	8.35 0.05	
3999.64*	9.17 0.03	8.50 0.03	7.84 0.03	
4001.55*	8.93 0.03	8.27 0.03	7.64 0.03	
4003.63	9.10 0.06	8.47 0.05	7.84 0.04	
4070.49	9.69 0.07	9.12 0.05	8.33 0.04	
4071.48	9.80 0.08	9.10 0.06	8.29 0.05	
4072.44	9.64 0.08	8.91 0.05	8.26 0.04	
4079.48*	9.55 0.03	8.90 0.03	8.20 0.03	
4080.41	9.63 0.11	8.87 0.05	8.27 0.06	
4082.46	9.55 0.08	8.86 0.07	8.09 0.09	
4083.40*	9.56 0.03	8.91 0.03	8.21 0.03	
4083.41	9.57 0.08	9.06 0.06	8.22 0.06	
4094.39	9.51 0.08	8.90 0.05	8.31 0.16	
4096.39	8.56 0.06	7.78 0.05	7.08 0.04	
4099.39	9.66 0.06	9.08 0.06	8.27 0.05	
4100.37	9.56 0.06	8.95 0.05	8.23 0.04	
4101.31	9.44 0.08	8.75 0.09	8.19 0.04	
4103.40	9.30 0.06	8.60 0.05	7.96 0.04	
4107.37	8.95 0.08	8.18 0.05	7.56 0.04	
4109.37	8.84 0.07	8.12 0.05	7.45 0.04	
4113.35	9.32 0.07	8.70 0.06	8.12 0.12	
4140.30*	8.92 0.03	8.17 0.03	7.54 0.03	
4141.29*	8.93 0.03	8.22 0.03	7.61 0.03	
4141.38*	8.89 0.03	8.22 0.03	7.59 0.03	
4171.25*	9.20 0.03	8.55 0.03	7.94 0.03	
4172.25*	9.09 0.03	8.41 0.03	7.80 0.03	
4173.26*	9.12 0.03	8.42 0.03	7.83 0.03	
4175.25*	9.31 0.03	8.60 0.03	7.94 0.03	
4176.25*	9.53 0.03	8.85 0.03	8.18 0.03	
4317.65*	9.11 0.03	8.44 0.03	7.85 0.03	
4346.61	9.39 0.07	8.79 0.05	8.11 0.05	
4347.64	9.38 0.06	8.64 0.06	8.02 0.05	
4348.62	9.19 0.06	8.48 0.06	7.83 0.04	
4349.62	9.16 0.06	8.52 0.05	7.89 0.04	
4360.65	9.49 0.07	8.88 0.06	8.19 0.05	
4362.64	9.31 0.07	8.64 0.06	8.02 0.05	
4363.64	9.42 0.07	8.79 0.06	8.14 0.05	
4429.51	9.27 0.06	8.57 0.06	7.84 0.04	
4434.52	9.30 0.06	8.58 0.05	7.96 0.04	
4435.49	9.10 0.06	8.36 0.05	7.70 0.04	
4439.44	9.07 0.06	8.40 0.05	7.73 0.04	
4440.38	9.20 0.06	8.58 0.05	7.88 0.04	
4441.42	9.22 0.06	8.59 0.05	7.84 0.04	
4442.43	9.36 0.06	8.69 0.05	7.97 0.04	
4444.42	9.31 0.06	8.66 0.05	7.96 0.04	
4445.45	9.29 0.06	8.56 0.05	7.89 0.04	
4446.48	9.04 0.06	8.32 0.05	7.59 0.04	
4447.45	9.30 0.06	8.61 0.05	7.93 0.04	
4448.43	9.31 0.07	8.64 0.05	7.92 0.04	
4449.43	8.96 0.06	8.23 0.05	7.52 0.05	
4450.45	8.84 0.06	8.05 0.05	7.27 0.05	
4452.40	9.35 0.06	8.65 0.05	7.96 0.04	
4454.47	9.26 0.06	8.59 0.06	7.96 0.05	
4455.30	9.26 0.06	8.60 0.05	7.96 0.04	
4457.38	9.38 0.07	8.80 0.06	8.06 0.05	
4458.47	9.53 0.07	8.79 0.06	8.09 0.04	
4459.33	9.47 0.07	8.72 0.06	8.14 0.05	
4466.37*	9.17 0.03	8.47 0.03	7.88 0.03	
4467.33*	9.19 0.03	8.45 0.03	7.85 0.03	7.18 0.03
4469.31*	9.28 0.03	8.56 0.03	7.92 0.03	7.14 0.03
4471.28	9.25 0.07	8.51 0.05	7.92 0.05	
4472.39	9.22 0.07	8.49 0.05	7.88 0.05	
4473.34	9.16 0.07	8.50 0.05	7.84 0.05	
4476.35	9.38 0.06	8.72 0.07	8.07 0.04	
4480.36*	9.03 0.03	8.36 0.03	7.80 0.03	
4481.29*	9.04 0.03	8.36 0.03	7.77 0.03	7.10 0.03
4482.29*	9.23 0.03	8.56 0.03	7.95 0.03	7.31 0.03
4483.23*	9.22 0.03	8.57 0.03	7.96 0.03	
4484.29*	9.23 0.03	8.52 0.03	7.93 0.03	7.32 0.03

* 1.88-m telescope observations.

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On Fig. 1, our data are folded onto the Giles *et al.*-suggested 11.8-day period, where the curve is their adopted lightcurve. For clarity the points are all displayed twice, covering two cycles. The more extensive data now available do not confirm their period. It is premature to use the IR data to define periodicities in the SS433 system, but it is important to see whether the well defined 165.5-day spectroscopic period, or other suggested spectroscopic periods are present in our data.

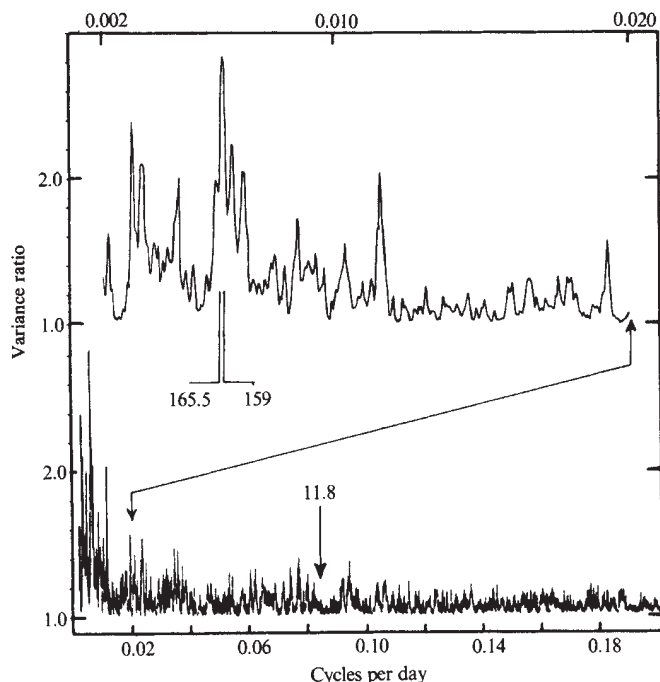


Fig. 2 The variance ratio using 5 bins for the SAAO data fainter than 7.6 K magnitude, is shown as a function of frequency, for periods in the range 5–500 days. The method is described in the text. The upper section shows the interval 50–500 days on an expanded frequency scale. Note the peak at 159 days and the absence of any peak at 11.8 days.

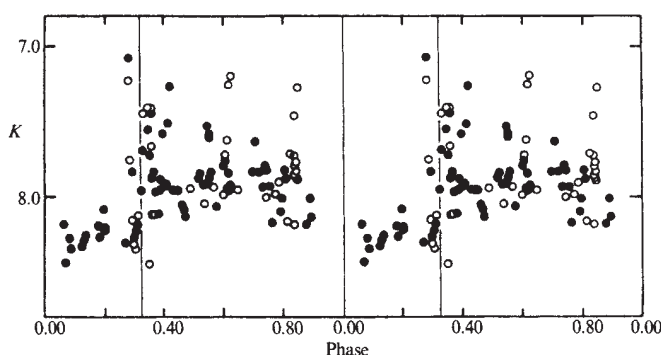


Fig. 3 The K magnitude as a function of phase for a 165.5-day period. Symbols are as in Fig. 1. The three vertical lines indicate the phases at which the radial velocities of the two high velocity emission line systems are equal. Phase 0.0 is on JD 2443718.9.

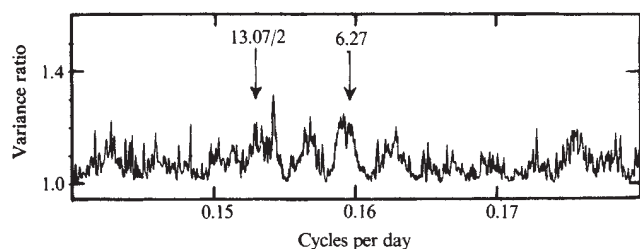


Fig. 4 The variance ratio is shown as a function of frequency for the faint SAAO data after removal of the 165.5-day period. The period interval is 5.5–7.0 days. Our weak peak at 6.27 days is marked as well as the position of Kemp *et al.* 13.07/2 period.

To avoid the effects of flaring we confine our search to data fainter than 7.6 in K . We use a method of periodic finding which, for each frequency, divides the data into phase bins and forms the ratio of the variance of the unbinned data to the weighted mean variance of the binned data. The weight is assigned according to the number of points in each bin. The method is especially suited to finding non-sinusoidal periodicities. Using this method with five bins and searching between 2 and 500 days as illustrated in Fig. 2 indicates that the most significant periodicity occurs at 159.0 days, with a variance ratio of 2.84. Snedecor's F test indicates that such a variance ratio has only a 1% probability of occurring by chance. Although it is difficult to assign quantitative errors to periods, the uncertainty can be judged from the breadth of the variance ratio peak which is ± 8 days at a variance ratio height of 2.0. This suggests that our periodicity is identical to the very well defined spectroscopic period of 165.5 days given by Ciatti *et al.*⁴ for the high-velocity emission line system. Figure 3 shows the data folded onto a 165.5-day period. Note that although there is no evidence for the 165.5-day period in the Giles *et al.* data alone, their data are not incompatible with this period. The three vertical lines in Fig. 3 indicate the phases at which the two high-velocity emission line velocity curves cross. In the jet model this corresponds to the phase when both jets are perpendicular to the line of sight. A striking feature of this plot is that the object is faint between phases 0.0 and 0.2. Note that flickering does not occur between these phases. The 165.5-day period has a range of 0.47 mag and shows excellent phase and amplitude agreement with the V magnitude periodicity found by Kemp *et al.*⁵.

Removing the 165.5-day periodicity from our data gives evidence for a 6.27-day period with a range of ~ 0.2 mag, an uncertainty of ± 0.03 days and a variance ratio of only 1.22 which is illustrated in Fig. 4. This period is very poorly defined but is probably to be identified with the 13.07-day double minimum periodicity found by Kemp *et al.* This in turn is identical with the 13.1-day periodic variation in the velocity of the stationary line spectrum found by Crampton *et al.*⁶ and thought to represent the motion of the central binary system in SS433. Confirmation of the reality of the 6-day period in our data and better phase coverage of the 165-day period must await the results of a further season's observing.

Figure 5 shows the $(J-H)$ colour index plotted as a function of the K magnitude and fitted by a least-squares line. This shows a definite trend towards redder colour, as the star becomes brighter contrary to the conclusions of Giles *et al.* However, this trend

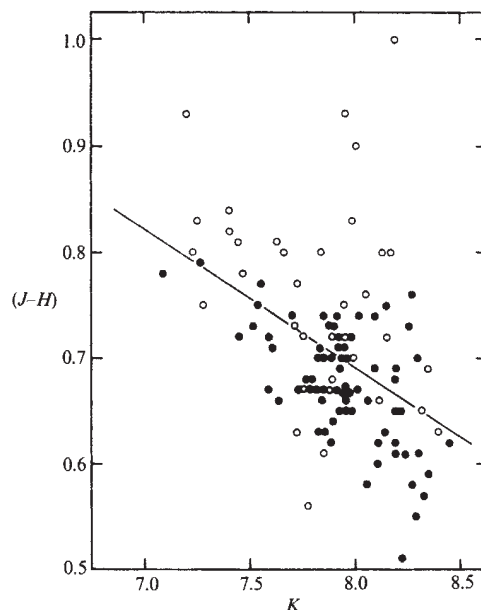


Fig. 5 The line, which is a least squares fit to all the data, illustrates the tendency of the $(J-H)$ colour to become redder as the star becomes brighter. Symbols are as in Fig. 1. The line has slope -0.129 ± 0.025 .

is not seen in ($H-K$). Wynn-Williams and Becklin⁷ find that in the 1–10- μm region SS433 becomes redder as it gets brighter, the trend increasing towards longer wavelengths. The ($J-H$) variation we observe may be related to the variation in the emission intensity and wavelength of H Paschen β , rather than to the changing optical depth in the free-free emission plasma, proposed by Wynn-Williams and Becklin to explain their data.

Murdin *et al.*⁸ suggest that the continuum radiation from a highly reddened O star contributes an important part of the JHK flux. If this were the case we might expect the 12-day period, which is considered the binary period of the system, to be the most conspicuous. It is also not clear how their model could explain the variation of ($J-H$) with magnitude. On the other hand, McAlary and McLaren⁹ consider that the $JHKL$ region is dominated by variable, line and optically thin free-free emission. The 165.5-day period presumably indicates that a major part of the JHK flux must originate from some part of the system involved with the jets or disk. The fact that the periodicities and trends of colour with magnitude are obscured by much 'noise' suggests that we are observing radiation generated by several mechanisms.

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1. Petterson, J. A. *Rev. at 23rd COSPAR Conf.* Budapest (1980).
2. Giles, A. B. *et al. Nature* **286**, 689–691 (1980).
3. Glass, I. S. *Mon. Not. astr. Soc. S. Afr.* **33**, 53–58 (1974).
4. Ciatti, F., Mammano, A. & Vittone, A. Preprint (Series N Osservatorio Astronomico di Padova 1980).
5. Kemp, J. C., Barbour, M. S., Kemp, G. N. & Hagood, D. M. *Vistas Astr.* (submitted).
6. Crampton, D., Cowley, A. P. & Hutchings, J. B. *Astrophys. J. Lett.* **235**, L131–L135 (1980).
7. Wynn-Williams, C. G. & Becklin, E. E. *Nature* **282**, 810–811 (1979).
8. Murdin, P., Clark, D. H. & Martin, P. G. *Mon. Not. R. astr. Soc.* **193**, 135–151 (1980).
9. McAlary, W. C. & McLaren, R. A. *Astrophys. J.* **240**, 853–858 (1980).

Thermal continuum emission from magnetic rotators in quasars

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Although accretion of gas onto supermassive black holes is a promising explanation of the energy source for quasars¹, magnetic rotator models² not only satisfy the large energy requirements, but they also can explain the X-ray emission from both quasars and Seyfert galaxies³. It is important to compare theoretical predictions of these two models with observations so that we may determine which model is correct. Shields⁴ has already done this for the black-hole accretion model; he finds that the thermal continuum emission from an accretion disk orbiting a $10^8 M_\odot$ black hole can explain the flat blue continuum of 3C273 and other quasars. I argue here that this flat continuum also finds a natural explanation on the magnetic-rotator model.

Supermassive rotators can be represented by $n = 3$ polytropes in which radiation pressure dominates the gas pressure throughout the body. Their properties have been discussed elsewhere^{5–7}. A polytrope of index n is a self-gravitating configuration in which the pressure distribution P obeys the law $P = K\rho^\gamma$, where ρ is the mass density distribution, K is a constant, and $\gamma = 1 + 1/n$ with $n > 0$. We are concerned with the (almost spherical) high-entropy configurations in equilibrium hydrogen-burning phases. In quasihydrostatic equilibrium, the central density is

$$\rho_c = 3A_n M / 4\pi R_p^3 \quad (1)$$

and the central pressure is

$$P_c = W_n GM^2 / R_p^4 \quad (2)$$

where M is the total mass and R_p is the polar radius (for polytropes of index $n = 3$, Chandrasekhar⁸ gives $A_n = 54.1825$ and $W_n = 11.05066$). As radiation pressure dominates the gas pressure, we may write the central pressure

$$P_{\text{rad}} = \frac{1}{3} a T_c^4 = W_n GM^2 / R_p^4 \quad (3)$$

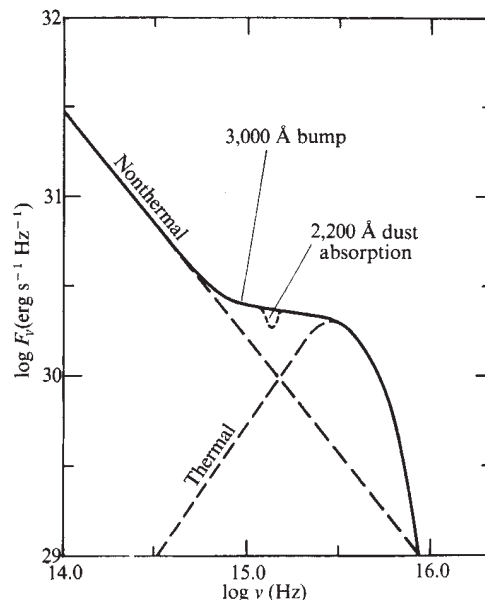


Fig. 1 The model continuum for $M_7 = 6$ and $Z_0 = 1$. The solid curve indicates the total continuum emission and a dust absorption feature near $\lambda = 2,200 \text{ \AA}$ is drawn. The nonthermal power $L_{\text{em}} = 5.8 \times 10^{48} \text{ erg s}^{-1}$ and the magnetic field $B \sim 10^3 \text{ G}$.

Hence the radius

$$R_p = \left(\frac{3W_n G}{a} \right)^{1/4} \frac{M^{1/2}}{T_c} \quad (4)$$

For supermassive rotators, the central temperature is fixed by the condition for thermal energy balance in which the rate of thermal energy loss equals the rate of nuclear energy generation on the carbon–nitrogen cycle. As the bulk of the nuclear reactions occur within a small temperature range, we may follow Schwarzschild⁹ and write the energy generation rate per unit mass

$$\varepsilon_{\text{cno}} = \varepsilon_1 \rho_c X X_{\text{cn}} T_c^{13} \quad (5)$$

where $X = 0.7$ is the hydrogen abundance, $X_{\text{cn}} = Z/3$ is the abundance of carbon and nitrogen nuclei, Z is the total metal abundance, and $\varepsilon_1 = 3 \times 10^{-91}$ in cgs units.

It has been shown^{5,6} that supermassive bodies in general would radiate their thermal energy at a rate approximating that of the Eddington luminosity $L_{\text{Edd}} = 4\pi c GM / \kappa$, where κ is the electron scattering opacity. Thus, the condition for thermal energy balance is approximately

$$\varepsilon_{\text{cno}} M = \varepsilon_1 X \frac{Z}{3} \rho_c M T_c^{13} = 4\pi c GM / \kappa \quad (6)$$

Using equations (1), (4), and (6) gives the central temperature

$$T_c = 3 \times 10^7 Z_0^{-1/16} \left(\frac{M}{M_\odot} \right)^{1/32} \text{ K} \quad (7)$$

where the reference abundance $Z_0 = Z/Z_{\text{cosmic}}$ and the cosmic abundance $Z_{\text{cosmic}} = 0.03$. Thus, the equilibrium polar radius is

$$R_p = 1.8 \times 10^{11} Z_0^{1/16} \left(\frac{M}{M_\odot} \right)^{15/32} \text{ cm} \quad (8)$$

from equations (4) and (7).

The equatorial radius R_* is slightly larger than R_p as a result of rotary and magnetic forces at the equator. But as (high entropy) magnetic rotators are almost spherical objects², we shall take $R_* = 1.5 R_p$ without any serious loss of generality. Thus

$$R_* = 5.25 \times 10^{14} Z_0^{1/16} M_7^{15/32} \text{ cm} \quad (9)$$

where M_7 is M measured in units of $10^7 M_\odot$.

A magnetic rotator (in its hydrogen-burning phase) would radiate a steady thermal luminosity at the Eddington rate

$$L_{\text{Edd}} = 4\pi c G M \kappa^{-1} = 1.25 \times 10^{45} M_7 \text{ erg s}^{-1} \quad (10)$$

for about 10^6 yr. The effective temperature of the rotator (that is, that temperature the rotator would have if it radiated like a black body) is

$$\theta_* = \left(\frac{L_{\text{Edd}}}{4\pi\sigma R_*^2} \right)^{1/4} = 5 \times 10^4 Z_0^{-1/32} M_7^{1/64} \text{ K} \quad (11)$$

where σ is Stefan's constant. Hence the thermal continuum emission peaks in the UV parts of the spectrum near the frequency

$$\nu_0 = 2.8 k \theta_* / h = 2.9 \times 10^{15} Z_0^{-1/32} M_7^{1/64} \text{ Hz} \quad (12)$$

Note that the mass-radius relation $R_* \propto M^{15/32}$ for super-massive rotators leaves the characteristic frequency $\nu_0 \propto L_{\text{Edd}} R_*^{-1/2} \propto M^{1/64}$ only weakly dependent on gross properties of the rotator (such as mass). This implies that ν_0 is almost the same $\sim 10^{15}$ Hz for a wide range of masses, a property that need not be the case for arbitrary gaseous configurations in quasistatic equilibrium.

If the rotator carries a magnetic-dipole field, then emission of magnetic-dipole radiation at the speed of light cylinder would accelerate particles to high energies there^{2,10,11}. This process occurs such that the rate of energy gain balances the synchrotron and Compton energy-loss rates¹¹. For a rotator spinning with angular velocity

$$\Omega_* \equiv \left(\frac{GM}{R_*^3} \right)^{1/2} = 3 \times 10^{-6} Z_0^{-3/32} M_7^{-13/64} \text{ s}^{-1} \quad (13)$$

the speed of light cylinder has radius

$$R_1 = \frac{c}{\Omega_*} = 9.8 \times 10^{15} Z_0^{3/64} M_7^{13/64} \text{ cm} \quad (14)$$

so emission variations would occur on time scales $\sim \Omega_*^{-1} \sim 4$ days. The energy flow across area $4\pi R_1^2$ carries an electromagnetic flux $\sim cB^2/4\pi$, where B is the magnetic field at radius R_1 . Hence the electromagnetic power

$$L_{\text{em}} \equiv B^2 c R_1^2 = 2.8 \times 10^{48} \left(\frac{B}{10^3 \text{ G}} \right)^2 Z_0^{3/32} M_7^{13/32} \text{ erg s}^{-1} \quad (15)$$

Neugebauer *et al.*¹² have reported the spectral energy distribution from 3,000 Å to 10 μm for about 40 quasars. In general the continua can be adequately represented by a power law $f_\nu \propto \nu^{-1}$ in the IR, but there are significant deviations from power-law behaviour in the optical and UV. I propose here that the enhanced blue continua of quasars is black-body emission from a central magnetic rotator; and that the characteristic frequency associated with this thermal emission is that given by equation (12).

It has been shown¹¹ that synchrotron emission from radius R_1 shows a power-law spectrum

$$F_s = 6 \times 10^{-21} N B \left(\frac{\nu_s}{\nu} \right)^\alpha \text{ erg s}^{-1} \text{ Hz}^{-1} \quad (16)$$

for $\alpha > 0$ and $\nu_s = 2.83 \times 10^6 B (E/mc^2)^2 \text{ Hz}$. N is the total number of cosmic-ray electrons with energy $E > mc^2$.

From the magnetic rotator I predict a black-body spectrum

$$F_\nu^* = 3.9 \times 10^{30} Z_0^{1/32} M_7^{63/64} \frac{(\nu/\nu_0)^3}{\exp(h\nu/k\theta_*) - 1} \text{ erg s}^{-1} \text{ Hz}^{-1} \quad (17)$$

Figure 1 shows the model composite spectrum (non-thermal and thermal) for a rotator with $M = 6 \times 10^7 M_\odot$ and $Z_0 = 1$. The non-thermal spectrum $F_s(\nu) = 5 \times 10^{29} (\nu_0/\nu)^{6/5} \text{ erg s}^{-1} \text{ Hz}^{-1}$, which corresponds to model parameters $\alpha = 6/5$, $\nu_s = \nu_0 = 3 \times 10^{15} \text{ Hz}$, $B \approx 10^3 \text{ G}$, $E = 10^3 mc^2 = 5 \times 10^8 \text{ eV}$, and $N \approx 8.3 \times 10^{46}$. The illustration clearly shows that the synchrotron spectrum dominates the IR, but the black-body emission dominates the UV near the Lyman continuum. On the

magnetic-rotator model it is the combination of nonthermal emission from the light cylinder and thermal emission from the rotator that produces a flattening of the continuum in the blue. The model also predicts that the spectrum of ionizing continua in quasars and Seyfert galaxies should closely follow a black-body law rather than the power-law behaviour ($F_\nu \propto \nu^{-1}$) assumed in most photoionization models.

Richstone and Schmidt¹³ reported a wave-like feature in the UV continuum of 85 quasars. There are spectral minima near 4,000 Å and 2,000 Å, and a continuum maximum at 3,000 Å. To interpret this behaviour on the present model, I propose that the minimum near 4,400 Å marks the onset of the thermal continuum excess itself (see Fig. 1) whereas the minimum near 2,000 Å is produced by absorbing dust particles surrounding the central rotator. Hence the intermediate emission excess should appear as a 3,000 Å bump in the spectrum. The characteristic frequency $\nu_0 \propto M^{1/64}$ associated with this bump is almost the same $\sim 10^{15} \text{ Hz}$ in quasars showing quite different redshifts. Such a uniform spectral property is unexplained on black-hole accretion models.

Figure 2 shows the model composite continuum in comparison with the near IR, optical, and UV flux data¹⁴ for the quasar 3C273. The magnetic rotator has mass $M = 7 \times 10^8 M_\odot$ and $Z_0 = 1$. The synchrotron spectrum is the same as that for Fig. 1, so the model parameters for 3C273 are $E = 10^3 mc^2$, $B \approx 10^3 \text{ G}$, and $N \approx 8 \times 10^{46}$. The power-law spectrum dominates the IR, but there is a large flux excess in the optical and UV. Although a continuum depression is observed near 2,000 Å, the model predicts that the continuum should rise into the far UV and then turn over at frequencies between $10^{15.5}$ and 10^{16} Hz . The actual depression near 2,000 Å might be caused by absorbing dust particles or by absorption lines produced by gaseous material.

Several authors^{14,15} agree that Balmer continuum emission from recombining ionized hydrogen cannot explain the bump. The same conclusion applies to two-photon continuum emission from H I gases. It has been suggested¹⁶ that the bump is caused by emission lines produced by dust grains surrounding the broad emission-line regions of quasars. Notice, however, that the 3,000 Å bump in the spectrum of 3C273 has a luminosity $\sim 9 \times 10^{45} \text{ erg s}^{-1}$. Such a high luminosity might require an anomalously large amount of dust that would be incompatible with *UBV* colours of quasars in general.

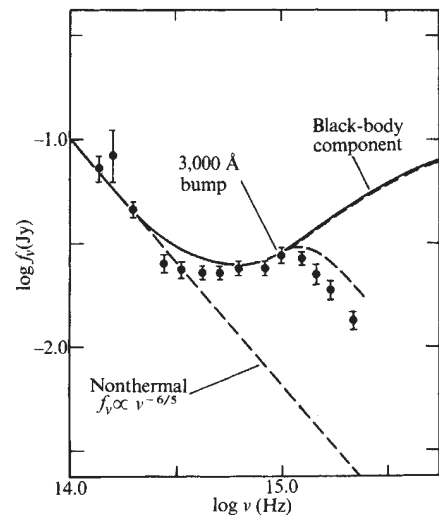


Fig. 2 The model continuum for 3C273. The solid curve indicates the model continuum flux for Hubble constant $H_0 = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and distance 512 Mpc at redshift 0.158 in an empty ($q_0 = 0$) Friedmann Universe. The dashed curve near 2,000 Å indicates the continuum depression that could be caused by absorbing gases or dust. The IR, optical, and UV flux data (without reddening corrections) were taken from ref. 14. The non-thermal power $L_{\text{em}} \approx 10^{49} \text{ erg s}^{-1}$ whereas the magnetic field $B \approx 10^3 \text{ G}$, $M = 7 \times 10^8 M_\odot$, $\nu_0 = 3 \times 10^{15} \text{ Hz}$.

Grandi and Phillips¹⁵ suggested that the bump is caused by emission-line blends (that is, Fe II multiplets). Although blends of emission lines are likely to make some contribution to the 3,000 Å bump, they alone cannot explain the whole behaviour of quasar spectra in the UV. Recent IUE data¹⁷ show that the flat spectrum continues until 10^{15.4} Hz, and thereafter the spectrum shows a marked steepening at higher frequencies. This spectral behaviour is found in many Seyfert galaxies and high-redshift quasars. The present model shows it in Fig. 1.

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1. Rees, M. *Ann. N.Y. Acad. Sci.* **302**, 613 (1977).
2. Ginzburg, V. L. & Ozernoy, L. M. *Astrophys. Space Sci.* **50**, 23 (1977).
3. Pacini, F. & Salvati, M. *Astrophys. J. Lett.* **225**, L99 (1978).
4. Shields, G. *Nature* **272**, 706 (1978).
5. Fowler, W. A. *Astrophys. J.* **144**, 180 (1966).
6. Fricke, K. J. *Astrophys. J.* **189**, 535 (1974).
7. Ozernoy, L. M. & Usov, V. V. *Astrophys. Space Sci.* **13**, 3 (1971).
8. Chandrasekhar, S. *Stellar Structure* (University of Chicago Press, 1938).
9. Schwarzschild, M. *Structure and Evolution of the Stars* (Dover, New York, 1958).
10. Ozernoy, L. M. & Usov, V. V. *Astrophys. Space Sci.* **25**, 149 (1973).
11. Cavaliere, A. & Morrison, P. *Astrophys. J. Lett.* **238**, L63 (1980).
12. Neugebauer, G., Oke, J. B., Becklin, E. E. & Matthews, K. *Astrophys. J.* **230**, 79 (1979).
13. Richstone, D. & Schmidt, M. *Astrophys. J.* **235**, 361 (1980).
14. Ulrich, M. H. *et al. Mon. Not. R. astr. Soc.* **192**, 561 (1980).
15. Grandi, S. A. & Phillips, M. M. *Astrophys. J.* **239**, 475 (1980).
16. Netzer, H. & Davidson, K. *Mon. Not. R. astr. Soc.* **187**, 871 (1979).
17. Green, R. F. *et al. Astrophys. J.* **239**, 483 (1980).

An Antarctic iron meteorite contains preterrestrial impact-produced diamond and lonsdaleite

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Of the many meteorites recovered so far from the Allan Hills, Antarctica, only nine have been irons. One of these, ALHA77283, contains troilite(FeS)-graphite(C)-schreibersite((Fe,Ni)₃P)-cohenite(Fe₃C) inclusions rich in the carbonado-type diamond-lonsdaleite 'nodules' previously described from the Canyon Diablo meteorite^{1,2}. The Canyon Diablo, Arizona, meteorite, the excavator of Meteor Crater, is the only other iron meteorite known to contain these high-pressure minerals, and their occurrence in that meteorite has been explained as the result of shock-induced transformation of graphite, most probably at the moment of terrestrial impact and disintegration of the projectile during crater formation³⁻⁶. The suggestion⁷ that formation was by high gravitational pressure has not been accepted. Virtually identical diamond-lonsdaleite-containing material in ALHA77283 occurs in a meteorite specimen with a well developed heat-altered zone produced by atmospheric ablation. It seems, therefore, that the diamond and lonsdaleite were present in the meteoroid before its final ablative passage through the atmosphere and soft landing on the ground. The shock event that produced these high pressure phases, therefore, must have taken place on its parent body or have been associated with the disruption of that body. Here we present the metallographic and X-ray diffraction data on which this conclusion is based.

ALHA77283 is a 10.5-kg carbon-rich octahedrite⁸ of chemical group IA, containing 7.33% Ni, 69 p.p.m. Ga, 230 p.p.m. Ge, and 2.2 p.p.m. Ir. It is similar in composition and structure to the Canyon Diablo meteorite and distinct from the other iron meteorites so far recovered from the Allan Hills. It has a generally rounded anterior surface that is suggestive of aerodynamic shaping during oriented atmospheric flight, and a slightly concave posterior surface (Fig. 1). Regmaglypts are not a prominent feature although there are fluted areas on one side of the

Table 1 X-ray powder diffraction data for diamond-lonsdaleite nodules (CuK α radiation; Ni filter)

Lonsdaleite + cubic diamond*		Film 7123 Allan Hills A77283		Film 6990 Canyon Diablo USNM 2262	
<i>d</i> (Å)	<i>I</i>	<i>d</i> (Å)	<i>I</i>	<i>d</i> (Å)	<i>I</i>
2.18	4	2.19	M	2.17	M
2.06	10 D	2.09	S D	2.09	S D
1.933	2	1.94	MW	1.94	W
1.50	1				
1.257	6 D	1.26	M D	1.26	M D
1.17	1	1.18	VVF		
1.075	3 D	1.08	W D	1.07	W D

D, Reflections coincident with those of cubic diamond; S, strong; M, medium; MW, medium-weak; W, weak; VVF, very, very faint.

* From ref. 2.

anterior surface near where it joins the posterior surface. Sectioning would be required to determine whether these are due to ablation or weathering. The specimen has been severely weathered, particularly on the posterior surface. Wind abrasion while on the ice also seems to have been important. Taenite (γ -FeNi, face-centred cubic) bands stand out in relief over much of the surface, revealing its internal octahedrite structure.

It was while cutting a median slice (Fig. 2) through the specimen that the presence of diamond was suspected. Cutting was slow, and the resulting surface revealed a cohenite-rich meteorite with troilite-graphite-schreibersite-cohenite inclusions containing black knobby protrusions up to ~0.5 mm in diameter, which protruded from narrow rims of black material of columnar structure that frequently separated troilite from schreibersite. These unusually hard inclusions prevented adequate polishing of the troilite inclusion and its immediate surroundings. Therefore, our observations on this association are tentative, but they do generally agree with the descriptions of the carbonado-type diamond-lonsdaleite 'nodules' previously reported in the Canyon Diablo meteorite¹⁻⁷.

Carbonado-like material was isolated from a small troilite inclusion on the back of the slice, area A in Fig. 2. The inclusion was dissolved in dilute hydrochloric acid, the insoluble residue treated several times with aqua regia, the light residue decanted from the heavy, and the heavy residue finally leached several times with boiling water. Part of this residue was examined by X-ray diffraction, revealing the presence of diamond, lonsdaleite, and a graphite-like phase. The diamond and lonsdaleite patterns are diffuse and the graphite pattern sharp. Diamond

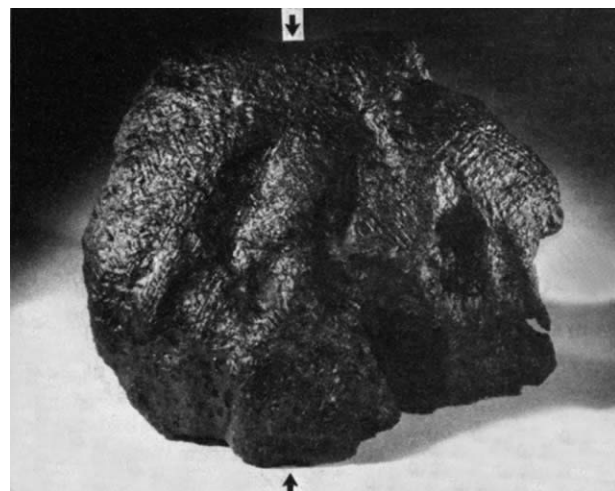


Fig. 1 Meteorite ALHA77283, showing the generally rounded anterior surface that has been weathered and abraded sufficiently so that taenite lamellae stand out in relief, revealing the internal Widmanstätten structure. The specimen is sitting on its more heavily corroded posterior surface. Arrows indicate the position of the slice shown in Fig. 2. Width of specimen is 16.5 cm.

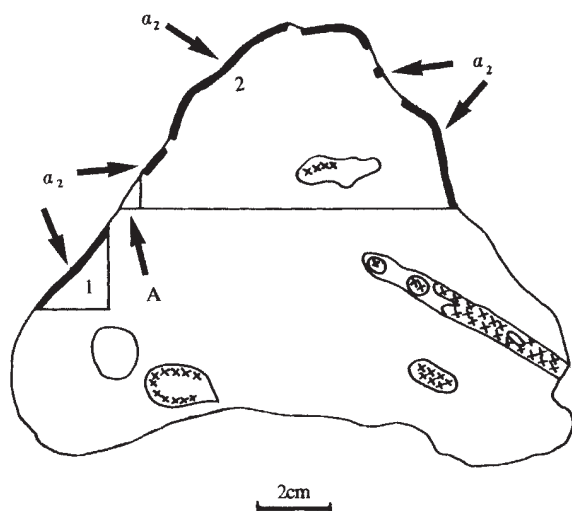


Fig. 2 Outline of slice through ALHA77283 perpendicular to the face shown in Fig. 1. Enclosed areas indicate troilite inclusions. $\times$, Distribution of graphite + diamond + lonsdaleite material identified visually on the rough cut surface. Areas 1 and 2 have been examined metallographically. Heavy black lines indicate presence of α_2 structure produced by ablative heating during atmospheric passage. The back side of area A contained a small troilite inclusion from which the material used for X-ray diffraction studies was obtained. The left edge in the sketch is the surface shown in Fig. 1.

and lonsdaleite are both present in appreciable amounts and are extremely fine-grained, with a grain size of the order of 100 Å. Preferred orientation is present in all three phases. Similar material was extracted from two previously-studied Canyon Diablo samples, USNM-1724 (refs 1, 4–6) and USNM-2262 (ref. 6). Both of these samples contained virtually identical assemblage of extremely fine-grained diamond, extremely fine-grained lonsdaleite, and coarser-grained graphite, with appreciable preferred orientation. These observations agree with previously reported results and are consistent with direct conversion of graphite to diamond and lonsdaleite by shock (Table 1).

Metallographic examination of polished sections (areas 1 and 2, Fig. 2) indicate that the specimen has been shock-loaded to moderate intensity^{9–11} and that the shock event(s) took place preterrestrially. The following observations were made away from the heat-altered zone at the edge of the specimen. The kamacite (α -FeNi, body-centred cubic) matrix is moderately clear, containing slightly distorted Neumann bands, and patchy ϵ transformation structure that is frequently associated with cohenite grains (Fig. 3a). Taenite and plessite are present and appear unaffected. The structure is dominated by cohenite that is cracked, frequently offset, and badly plucked. The cohenite appears to be single crystal with a suggestion of undulatory extinction, and neither decomposition to graphite nor diffusion borders in kamacite were seen. Grain-boundary schreibersite is present, as are small schreibersites associated with cohenites. Rhabdites were not observed. Several small schreibersites at cohenite borders seem to have been partially melted, and small areas of adjacent kamacite converted to martensite. Some of these melted schreibersites are associated with areas of transformation within the cohenite, but other areas of transformed cohenite are seen without schreibersite¹². No troilite sufficiently well polished was available for examination. Weathering within the structure is light and generally associated with cracked cohenite or schreibersite inclusions. These observations suggest shock loading in the moderate range of Heymann *et al.*⁵, well above 200 kbar but less than the 750 kbar required to transform kamacite to martensite. The two specimens of the Canyon Diablo material we used for X-ray examination were also shocked in the moderate range⁶. These studies are continuing and will be published in detail elsewhere.

Metallographic examination revealed a well developed heat-altered zone over most of the available sample edge (Fig. 2), a structure that forms during ablative passage through the atmosphere^{10,11}. In this zone the kamacite has been transformed to martensite (α_2 , Fig. 3b), taenite has been rendered clear, schreibersite melted to a eutectic, and carbon diffusion zones produced around cohenite. Terrestrial oxides subsequently formed at the outer edge. The martensite zone is up to 1.5 mm thick, and where it is thin or absent it seems to have been lost to weathering. No remnant fusion crust was observed.

The presence of a heat-altered zone on ALHA77283 demonstrates that it entered the atmosphere from space, was ablatively decelerated, and landed softly. Therefore, the shock loading that produced the observed metallographic changes and the diamond and lonsdaleite must have taken place before atmospheric entry, but after Widmanstätten pattern formation. The most reasonable assumption is that these features were produced in the meteoroid at the time of parent body breakup or on subsequent collisions in space.

The close similarity between ALHA77283 and diamond-lonsdaleite-bearing Canyon Diablo specimens is striking.

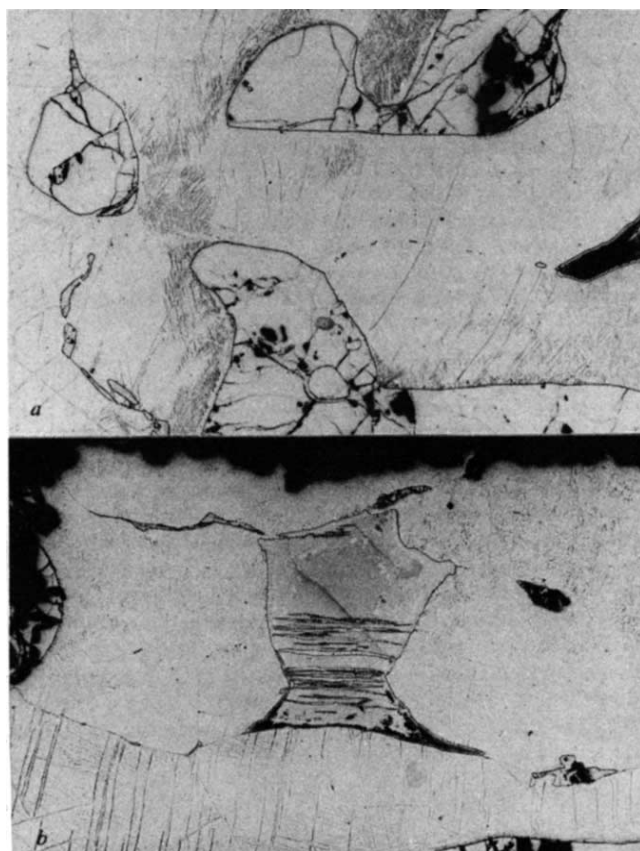


Fig. 3 Photomicrographs of metallographic structures revealed by acid etching of polished section 2 of meteorite ALHA77283. *a*, A 2.2-mm wide area at least 2 cm away from the nearest edge. The three large cracked inclusions are cohenite, the string of small inclusions at the lower left are schreibersite, and the dark inclusion at the middle right is a taenite-plessite area. The matrix is kamacite, containing distorted Neumann bands and patches of feathery ϵ transformation structure, particularly associated with edges of cohenite grains. The presence of the ϵ transformation structure establishes shock loading above 200 kbar. *b*, A 3.5-mm wide area of structure at the edge of the specimen. The ablation-produced heat-altered zone is at the top and is ~1 mm wide. The kamacite has been converted to granular martensite (α_2), the string of schreibersites at the top have undergone incipient melting at their edges, and the top of the taenite-plessite area does not colour when etched. In the less intensely heated area at the bottom, Neumann bands have been retained in the kamacite and the taenite-plessite colours on etching. These features are the consequence of a brief and steep thermal gradient produced by ablative heating.

Meteoroids have complex histories and shock events happen at various stages in their development. In certain circumstances sequential opportunities would be provided for shock to act on susceptible mineral associations to produce these diamond assemblages. This has been postulated as having taken place at the time of terrestrial impact for the canyon Diablo meteorite⁴⁻⁶. In the ALHA77283 meteoroid, however, diamond formation almost certainly took place preterrestrially. That this also happened in the Canyon Diablo meteoroid cannot be ruled out.

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1. Ksanda, C. J. & Henderson, E. P. *Am. Miner.* **24**, 677-680 (1939).
2. Frondel, C. & Marvin, U. B. *Nature* **214**, 587-589 (1967).
3. Nüniger, H. H. *Arizona's Meteorite Crater* (American Meteorite Museum, Arizona, 1956).
4. Lipschutz, M. E. & Anders, E. *Geochim. cosmochim. Acta* **24**, 83-105 (1961).
5. Heymann, D., Lipschutz, M. E., Nielsen, B. & Anders, E. *J. geophys. Res.* **71**, 619-641 (1966).
6. Anders, E. & Lipschutz, M. E. *J. geophys. Res.* **71**, 643-661 (1966).
7. Carter, N. L. & Kennedy, G. C. *J. geophys. Res.* **69**, 2403-2421 (1964).
8. Clarke, R. S. Jr., Jarosewich, E., Goldstein, J. I. & Baedeker, P. A. *Meteoritics* **15**, 273-274 (1980).
9. Jain, A. V. & M. E. Lipschutz. *Chem. Erde* **30**, 199-215 (1971).
10. Axon, H. J. & Couper, W. R. D. *Mineral. Mag.* **40**, 827-841 (1976).
11. Buchwald, V. F. *Handbook of Iron Meteorites* (University of California Press, 1975).
12. Axon, H. J., Couper, W. R. D. & Kinder, J. *Nature* **267**, 414-415 (1977).

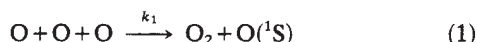
Excitation of the green line in the night airglow

R. D. Kenner, E. A. Ogryzlo & P. T. Wassell

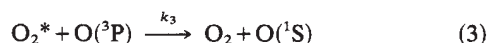
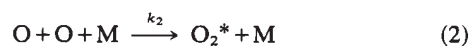
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In directions away from the Milky Way, most of the visible light from the night sky comes from a glowing layer of gas at an altitude of ~97 km. About 20% of this light is concentrated in a sharp green line at 557.7 nm which is a prominent feature of the visible portion of the night airglow. This emission comes from an excited oxygen atom ($O(^1S)$), but the mechanism by which this state is populated has been the subject of controversy ever since the emission was first identified in 1925¹. We present here new laboratory evidence concerning the processes governing the 557.7-nm emission of $O(^1S)$.

Because the maximum emission intensity of $O(^1S)$ occurs at an altitude where the oxygen atom concentration is at a maximum, it was assumed between 1931 and 1961 that the excitation occurs in the reaction²:



which has been called the Chapman reaction. To explain the absence of any green line emission in a laboratory system containing oxygen atoms, Barth³ proposed an indirect mechanism:



called the Barth mechanism. Laboratory studies have not yet provided clear evidence for or against either mechanism, although rocket measurements on the 557.7-nm intensity profile⁴⁻⁶ apparently favour the Barth mechanism, and the theoretical considerations of Bates⁷ suggest that the Chapman mechanism would be less efficient at producing $O(^1S)$. One complicating feature has been the magnitude of the quenching constant for the reaction:

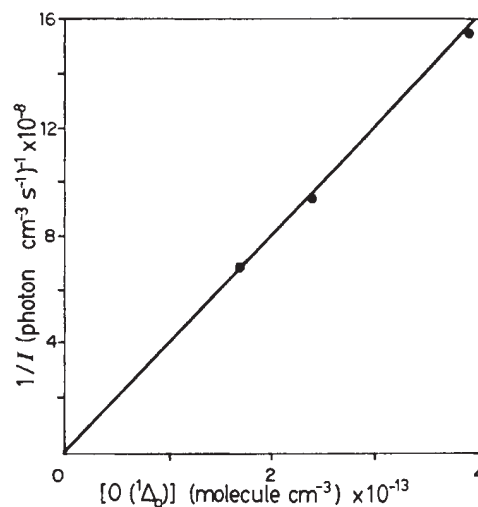


Fig. 1 The $O_2(^1\Delta_g)$ dependence of the 557.7-nm emission intensity (I). $[Ar] = 1.3 \times 10^{17}$ atom cm^{-3} , $[O] = 1.2 \times 10^{15}$ atom cm^{-3} .

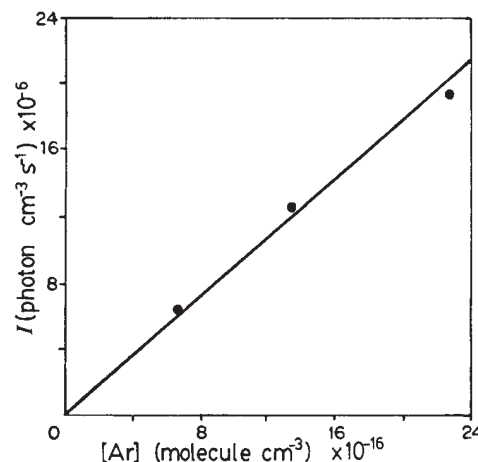
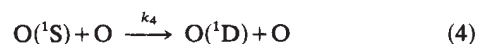


Fig. 2 The pressure dependence of the 557.7-nm emission intensity (I) over the range of 2-6 torr Ar. $[O_2(^1\Delta_g)] = 2 \times 10^{13}$ molecule cm^{-3} , $[O] = 7 \times 10^{14}$ atom cm^{-3} .



Reported values for k_4 have risen dramatically over the years⁸: 2.7×10^{-15} (1961), 1.8×10^{-13} (1966), 7.5×10^{-12} (1972) and 2×10^{-11} (1976) (in units of cm^3 per molecule per s). However, Slinger has found (personal communication) that the later high values are erroneous, with the error arising because of the presence of another quencher in these systems which is most probably $O_2(^1\Delta_g)$. The data presented here support this suggestion.

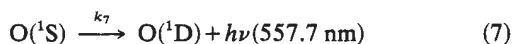
We prepared a stream of oxygen atoms by discharging a mixture of oxygen and argon, and passing the Ar- O_2 -O mixture into a 5-l bulb. The oxygen atom concentration was obtained by monitoring the visible NO_2 emission when known quantities of NO were added⁹. The $O_2(^1\Delta_g)$ concentration was calculated from the emission intensity at 1,270 nm using a McPherson 0.3-m monochromator and a dry ice-cooled lead sulphide detector. The 557.7-nm emission was obtained by scanning the peak with a 1-m Spex monochromator.

The results of our measurements of the intensity (I) of the 557.7-nm emission as a function of $O_2(^1\Delta_g)$, Ar and O are contained in Figs 1-3. These show an inverse dependence on the $O_2(^1\Delta_g)$ concentration, a first-order dependence on the total particle concentration (principally Ar in this case) and a second-order dependence on the oxygen atom concentration. These experimental results can be summarized in the equation:

$$I(557.7 \text{ nm}) = \frac{k[\text{O}]^2[\text{M}]}{[\text{O}_2(^1\Delta_g)]} \quad (5)$$

with the composite rate constant $k = 2.7 \times 10^{-27} \text{ cm}^3$ per molecule per s.

The rate law represented by equation (5) is not consistent with the simple Chapman reaction (equation (1)) but would result from the following 'Barth'-type mechanism, that is, formation by equations (2) and (3) followed by:



With standard steady-state assumptions, the composite rate constant in equation (5) is given by:

$$k = \frac{k_2 k_3 k_7}{k_5 k_6}$$

Although the rate of reaction (6) cannot be determined independently in our system, the relative efficiencies of O_2 and $\text{O}_2(^1\Delta_g)$ can be determined by adding O_2 to the gas stream. In the analysis we must add the reaction:

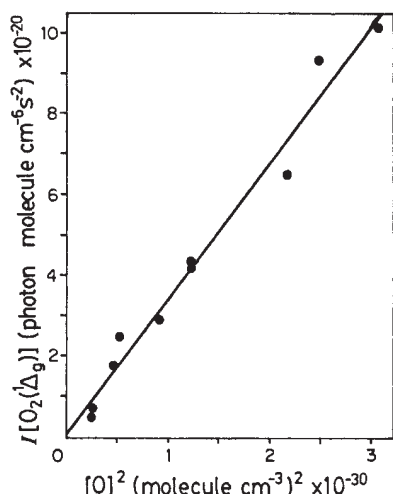
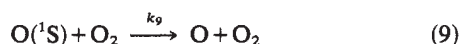


Fig. 3 The atomic oxygen dependence of the 557.7-nm emission intensity (I), corrected for different $[\text{O}_2(^1\Delta_g)]$. $[\text{Ar}] = 1.3 \times 10^{17} \text{ atom cm}^{-3}$.

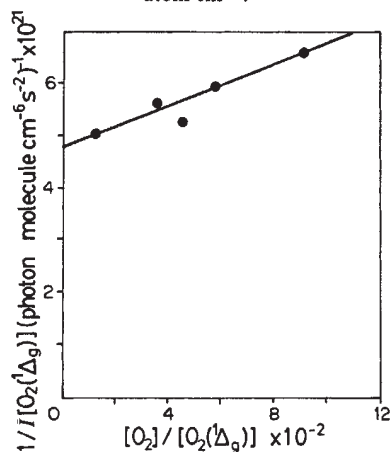


Fig. 4 The effect of the addition of O_2 on the 557.7-nm emission intensity as a function of $[\text{O}_2(^1\Delta_g)]$. $[\text{O}] = 7 \times 10^{14} \text{ atom cm}^{-3}$, $[\text{Ar}] = 1.3 \times 10^{17} \text{ atom cm}^{-3}$.

to the above reaction scheme. With standard steady-state assumptions for O_2^* and $\text{O}(^1\text{S})$ the rate equation can be written:

$$\frac{[\text{O}]^2[\text{M}]}{I(557.7 \text{ nm})[\text{O}_2(^1\Delta_g)]} = \frac{k_6 k_5}{k_3 k_2 k_7} + \frac{k_5 k_9 [\text{O}_2]}{k_3 k_2 k_7 [\text{O}_2(^1\Delta_g)]} \quad (10)$$

A plot of the left-hand side of equation (10) against $[\text{O}_2]/[\text{O}_2(^1\Delta_g)]$ should yield a straight line with intercept/slope = k_6/k_9 . Such a plot (with $[\text{O}]$ and $[\text{M}]$ constant) is shown in Fig. 4, from which $k_6/k_9 = 2.5 \times 10^3$. As $k_9(298 \text{ K}) = 2.2 \times 10^{-13} \text{ cm}^3$ per molecule per s (ref. 10) our results yield $k_6(298 \text{ K}) = 5.5 \times 10^{-10} \text{ cm}^3$ per molecule per s. This very large rate constant is comparable with that reported for the quenching of $\text{O}(^1\text{S})$ by O_3 (ref. 11).

Although quenching of $\text{O}(^1\text{S})$ by $\text{O}_2(^1\Delta_g)$ clearly dominates the 557.7-nm emission in the laboratory, it is difficult to see it playing a significant part in the upper atmosphere. From the 60 k R emission from $\text{O}_2(^1\Delta_g)$ in the 97-km region one can estimate a $\text{O}_2(^1\Delta_g)$ concentration of $\sim 1.8 \times 10^8 \text{ molecule cm}^{-3}$. Combining this with k_6 yields a removal rate an order of magnitude slower than the radiative decay rate of $\text{O}(^1\text{S})$, which is 1.065 s^{-1} .

We conclude that the recombination of oxygen atoms leads to the excitation of $\text{O}(^1\text{S})$ through a Barth-type mechanism in which the intermediate O_2^* remains to be identified. The quenching of $\text{O}(^1\text{S})$ by $\text{O}_2(^1\Delta_g)$ occurs at collision frequency and probably represents a very efficient energy-pooling process¹² which results in the dissociation of $\text{O}_2(^1\Delta_g)$.

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Received 11 March; accepted 25 March 1981.

1. McLennan, J. C. & Shrum, G. M. *Proc. R. Soc. A* **108**, 501–512 (1925).
2. Chapman, S. *Proc. R. Soc. A* **132**, 353–374 (1931).
3. Barth, C. A. J. *geophys. Res.* **67**, 1628 (1961).
4. Slanger, T. G. & Black, G. *Planet. Space Sci.* **25**, 79–88 (1977).
5. Thomas, L., Greer, R. G. H. & Dickinson, P. H. G. *Planet. Space Sci.* **27**, 925–931 (1979).
6. Witt, G., Stegman, J., Solheim, B. H. & Llewellyn, E. J. *Planet. Space Sci.* **27**, 341–350 (1979).
7. Bates, D. R. *Planet. Space Sci.* **27**, 717–718 (1979).
8. Bates, D. R. *Planet. Space Sci.* **26**, 897–912 (1978).
9. Sutoh, M., Morioka, Y. & Nakamura, M. *J. chem. Phys.* **72**, 20–24 (1980).
10. Slanger, T. G., Wood, B. J. & Black, G. *Chem. Phys. Lett.* **17**, 401–403 (1972).
11. Atkinson, R. & Welge, K. H. *J. chem. Phys.* **57**, 3689–3693 (1972).
12. Arnold, S. J., Finlayson, N. & Ogryzlo, E. A. *J. chem. Phys.* **44**, 2529–2530 (1966).

Porous titania glass as a photocatalyst for hydrogen production from water

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The rutile modification of TiO_2 in the form of a powder suspended in water shows promise as a photocatalyst for the production of hydrogen from water^{1,2}. Although hydrogen production was reported³ from pure rutile powder suspended in an aqueous solution, subsequent work^{1,2} revealed that sustained hydrogen production required Pt or RuO_2 on the rutile surface. This limiting usefulness of crystalline rutile in photocatalytic applications has prompted us to investigate the photocatalytic properties of amorphous titania in the form of a porous glass. Here we report an inexpensive method of forming monolithic porous titania glass (PTG) and some properties that make it uniquely suited to photocatalytic applications such as its inexpensive fabrication, its high surface area, and its spontaneous photoformation of H_2 from water without the need for precious metals.

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Table 1 Comparison of hydrogen production rates from PTG with those obtained by other methods

Catalyst and solution		Form of catalyst	Amount of catalyst (g)	Rate of H ₂ production (μmol h ⁻¹)	Ref.
SrTiO ₃	Conc. NaOH	Single crystal	—	0.1	17
TiO ₂	Sugar	Powder	0.3	0.01	18
TiO ₂ /RuO ₂ /Pt	Sugar		0.3	14.0	18
TiO ₂ /RuO ₂ /Pt	H ₂ O		0.3	0.02	18
TiO ₂ /RuO ₂ /Pt	Sugar + 6 M NaOH		0.3	17.0	18
TiO ₂	50% MeOH		0.3	27.0	19
TiO ₂ /RuO ₂ /Pt	50% MeOH	Glass†	0.3	520.0	19
PTG	H ₂ O		1.5	~0.01	This work
PTG	50% KOH		1.5	0.03	
PTG	MeOH + 50% KOH		1.5	6.0	
PTG/Cu	MeOH + 50% KOH		1.5	27.0	
PTGB*	50% KOH	Glass‡	1.5	Trace	This work
PTGB*	MeOH + 50% KOH		1.5	14.0	
PTGB/Cu	MeOH + 50% KOH	Glass‡	1.5	280	This work
Translucent aerogel†	H ₂ O	Chunks	1.5	912	

*PTGB, porous titania glass treated with base: soaked in 50% KOH, dried at 80 °C overnight.

†TiO₂ produced by hydrolysing Ti(OiPr)₄ and autoclaving the gel at 270 °C.

‡Particles averaging 5 mm in diameter, 1 mm thick.

Although titania gels were first reported in 1823⁴, no successful attempt to dehydrate the gel to a single piece of porous glass has been reported. Titania gels were originally formed using inorganic methods: adding TiCl₄ to concentrated HCl followed by partial neutralization⁵, or adding acid to a sodium titanate solution⁶. Inorganic methods produce salts which must be removed by dialysis before the sol-gel transition will occur⁶. In an effort to eliminate the presence of salts and hence the need for dialysis, organo-titanium reagents have been used to form titania gels. The hydrolysis of titanium alkoxides results in the formation of amorphous powders with large surface areas, but not monolithic (pieces larger than 1 cm²) samples⁷. More recently, monolithic glasses containing titania have been reported⁸. These mixed titania-silica glasses are formed by polymerizing a mixture of silicon and titanium alkoxides. In view of the low cost of TiCl₄, we modified an inorganic method of producing PTG in the form of flat wafers 0.5–1 mm thick and up to 3.5 cm in diameter⁹.

The dropwise addition of 6 mol of water to 1 mol of TiCl₄ at low temperatures results in a clear, colourless solution. Up to 60% of the Cl⁻ in this solution can be removed as HCl (corresponding to 2.4 mol of HCl per mol of TiCl₄) by low pressure, room temperature distillation. The viscous residue can be redissolved in water. Careful addition of KOH to this solution results in the neutralization of 0.2 mol HCl; adding more KOH results in permanent turbidity. From this partially neutralized solution, a stock solution containing 0.35 mol Ti/L, can be kept for months at 4 °C, but becomes turbid on standing at room temperature for a few days. Dialysis of the stock solution results in a rapid increase in viscosity after 3–4 h; in 12 h the dialysed solution sets to a clear gel. After drying for 10 days at room temperature, the gel contracts to 30% of its original volume. Dehydration of this air-dried gel at 160 °C results in a weight loss of 22%, but no change in size; this high temperature dehydration gives a microporous structure. Additional heating above 160 °C results in a further 2% weight loss, followed by the onset of crystallization to anatase at 350 °C. A similar crystalline phase change occurring at 300 °C has been reported for a precipitated amorphous titania powder¹⁰.

The IR spectrum of PTG outgassed at 220 °C (0.01 torr) for 10 h is shown in Fig. 1a. Sharp bands in the 3,700 cm⁻¹ region are assigned to surface O–H stretching frequencies unperturbed by hydrogen bonding¹¹. In this region PTG shows two bands, at

3,659 and 3,638 cm⁻¹; quite different from either rutile (3,680 cm⁻¹)¹² or anatase (3,715, 3,675 cm⁻¹)¹³. Heating the glass to higher temperatures results in the loss of both bands due to surface dehydroxylation (Fig. 1a–c). Deuteration of the surface by exposure to D₂O liquid followed by outgassing at 330 °C (Fig. 1d) gives a ν_{OH}/ν_{OD} ratio 1.36 compared with the expected 1.37 isotope shift for an isolated OH group. The BET surface area decreases from 490 m² g⁻¹ for a sample heated at 300 °C to 146 m² g⁻¹ for samples heated at 350 °C. The crystallization to anatase (as determined by X-ray powder patterns) that occurs in the 300–350 °C range not only accounts for the decrease in surface area, but also renders the glass visibly opaque. Surface area changes, weight loss, and crystallization behaviour determined for PTG are all very similar to amorphous titania prepared by precipitation from solution by base addition¹⁴. PTG dehydrated at temperatures < 350 °C has a pale yellow colour—this colour and ESR data indicate the presence of surface Ti(IV) peroxy species. The ESR signals observed on PTG are the same as those observed in anatase treated with H₂O₂ and assigned to O₂⁻ species¹⁵.

Rutile produces hydrogen photocatalytically from water when exposed to visible light only when the rutile surface also contains Pt or RuO₂ (refs. 1,2,16). This can be explained by the close proximity of the conduction band edge of rutile to the reduction potential of water (Fig. 2), necessitating the presence of precious metals to reduce the overpotential for H₂ evolution at the surface. On the other hand, the upward displacement of the conduction band edge in SrTiO₃ above the reduction potential of water (Fig. 2) may explain why precious metals are not necessary for photocatalytic hydrogen production in SrTiO₃ (ref. 17). As PTG, like SrTiO₃, will produce H₂ from water without the intervention of precious metals, we conclude the PTG must have a bandgap structure similar to SrTiO₃. Furthermore, SrTiO₃ and the anatase modification of TiO₂ have identical bandgaps on 3.2 eV. From X-ray powder studies, PTG closely resembles anatase, being transformed into anatase at 350 °C.

When untreated PTG is immersed in water and illuminated with visible light from a 2,500 W high pressure Xenon lamp operated at 65% of rated power to match the solar spectrum in frequency distribution and intensity, small amounts of H₂ are produced as measured by gas chromatography (2 m, 5 Å molecular sieve column, Argon carrier gas with thermal conductivity detector). When PTG is immersed in 50% KOH and irradiated, it turns intense blue-black in colour and the rate of H₂ production increases slightly. The blue-black colour is due to the reduction of Ti(IV) to blue-black Ti(III). The intense colour is

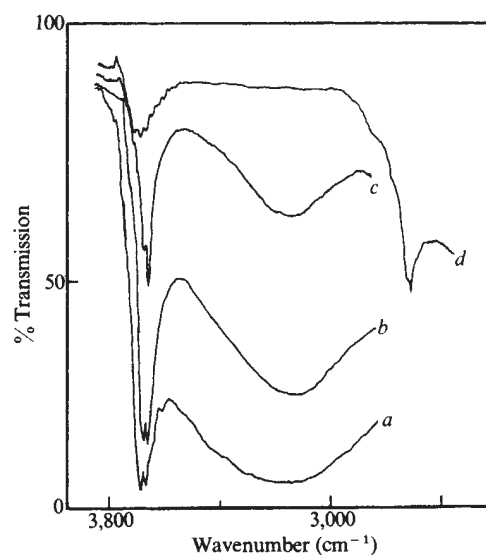


Fig. 1 IR spectra of titania glass (0.8 mm thick) after outgassing at: a, 220 °C for 10 h; b, 330 °C for 10 h; c, 380 °C for 8 h; d, exchange with D₂O (liquid) followed by 8 h outgassing at 330 °C.

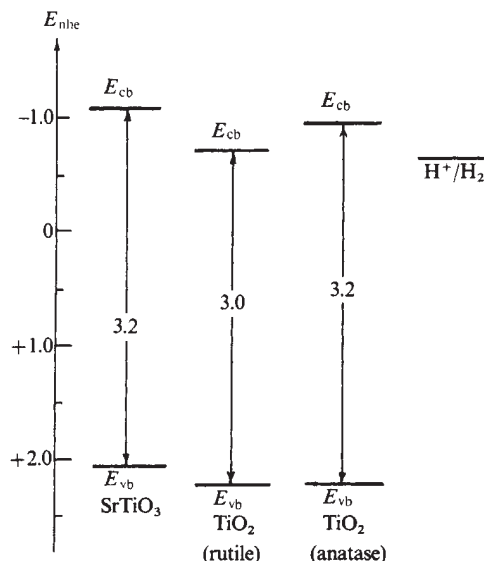


Fig. 2 A comparison of bandgap energy positions in anatase, rutile and SrTiO_3 relative to the reduction potential of water (H^+/H_2). Energy levels are shown for pH 7.

stable over several days as long as there is no contact with O_2 , and will remain for about a week in the air if totally immersed in solution. By providing an oxidizable substance such as methanol, the colour will disappear more rapidly and H_2 production will increase dramatically. Irradiation experiments with CH_3OH in D_2O produced D_2 and not H_2 ; with added CD_3OH in H_2O only H_2 was formed. Thus H_2 produced in this way comes from water and not the alcohol. A further addition of 0.3 mol of Cu(II) in the form of CuCl_2 solution increases the rate of H_2 production further. These results are summarized and compared with previous work in Table 1.

If PTG is soaked in 50% KOH and then dried before exposing to light, photocatalytic H_2 production is doubled with respect to untreated PTG. The addition of the Cu(II) solution to PTGB (PTG base treated) increases the rate of H_2 evolution by an order of magnitude. The mechanism of Cu enhancement is not entirely clear. The Cu(II) is removed from solution, probably becoming incorporated into the TiO_2 structure. The PTG or PTGB becomes a purplish colour on exposure to light, indicating that copper is in the form of the free metal or Cu_2O ; the purple colour resulting from the combination of the red of Cu metal or Cu(I) oxide and the blue-black of Ti(III) . The Cu-treated glasses revert to a pale greenish yellow on exposure to air.

The data summarized in Table 1 show some trends worth noting: the rate of H_2 production can be increased by the presence of oxidizable substances such as sugar or methanol and/or the presence of strong base such as NaOH or KOH. As the catalysts are of different forms, a direct comparison may not be valid as the mechanisms for the H_2 production may depend on the form of the catalyst, surface area and light intensity.

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1. Sato, S. & White, J. M. *Chem. Phys. Lett.* **72**, 83–86 (1980).
2. Kawai, T. & Sakata, T. *Chem. Phys. Lett.* **72**, 87–89 (1980).
3. Schrauzer, G. M. & Guth, T. D. *J. Am. chem. Soc.* **99**, 7189–7193 (1977).
4. Rose, H. *Liebigs Ann.* **73**, 76 (1923).
5. Weiser, H. B. *J. phys. Chem.* **46**, 1051–1059 (1942).
6. Klosky, S. & Marzano, C. *J. phys. Chem.* **29**, 1125–1128 (1925).
7. Harris, M. R. & Whitaker, G. *J. appl. Chem.* **12**, 490–494 (1962).
8. Yoldas, B. E. *J. Mater. Sci.* **14**, 1843–1849 (1979).
9. US Patent Pending.
10. Vivien, D., Levage, J. & Mazieres, C. *J. chim. Phys.* **67**, 199–204 (1979).
11. Yates, D. J. *J. phys. Chem.* **65**, 746–753 (1961).
12. Jackson, P. & Parfitt, G. D. *Trans. Faraday Soc.* **67**, 2469–2483 (1971).
13. Primet, M., Pichat, P. & Mathieu, M.-V. *J. phys. Chem.* **75**, 1216–1220 (1971).
14. El-Akkad, T. M. *J. Coll. Interface Sci.* **76**, 67–73 (1980).
15. Munuera, G., Gonaes-elipe, A. R., Soria, J. & Soz, J. *JCS Faraday I* **76**, 1535–1546 (1980).

16. Borgarello, E., Kiwi, J., Pelizzette, E., Visca, M. & Grätzel, M. *Nature* **289**, 158–159 (1981).
17. Wagner, F. T. & Somarjai, G. A. *Nature* **285**, 559–560 (1980); *J. Am. chem. Soc.* **102**, 5494–5502 (1980).
18. Kawai, T. & Sakata, T. *Nature* **286**, 474–476 (1980).
19. Kawai, T. & Sakata, T. *JCS Chem. Commun.* 694–695 (1980).

Scanning electron-stimulated desorption microscopy of model-biological surfaces

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Electron-stimulated desorption (ESD) of ions and neutrals from surfaces has been used to study chemisorption in many simple gas–solid systems^{1,2}, but there has been much less effort devoted to ESD studies with significant spatial resolution^{3–7}. ESD contrasts with scanning electron microscopy because ESD signals depend sensitively on the coverage and binding of an adsorbed species⁸. Applying scanning ESD to biological surfaces permits the surface chemical variations to be mapped using simple low mass adsorbates⁹ in comparison with conventional electron microscopy of biological matters which relies on heavy-metal stain technology⁹. We show here how scanning ESD can complement scanning Auger electron microscopy in mapping the surface chemistry of biological substrates. A model biological substrate constructed from a deposited synthetic polypeptide monolayer (polymethyl-glutamate) is used as the controlled sample surface. Micrographs display a spatial variation in H^+ desorption with a resolution of 10^{-4} cm. We also discuss limitations to resolution as dictated by signal-to-noise considerations. The contrast mechanism would be useful for studying the important biological surface problems such as membrane morphology, cell-interfacial composition, and cell-to-cell adhesion.

The micrographs were made using a scanning Auger microprobe¹⁰. A scanning electron gun, consisting of a LaB₆ source, electrostatic lenses and deflection plates, is concentrically mounted within a cylindrical mirror electron energy analyser. The electron gun can project a beam with a diameter of 1,600 Å with currents of 10^{-8} – 10^{-7} A onto the target. With a defocused beam (diameter $\sim 1\mu\text{m}$) larger currents ($\sim 10^{-6}$ A) can be projected and scanning Auger micrographs (SAM) and standard secondary electron micrographs (SEM) can be obtained¹⁰. Ions desorbed by the electron beam striking the target are energy-analysed electrostatically¹¹ and then mass-analysed with a quadrupole mass filter before detection with a CuBe multiplier. The amplified output of this detector was used for intensity modulation of an oscilloscope to produce scanning desorption micrographs (SDM). The entire apparatus is housed in a stainless steel ultrahigh vacuum chamber pumped by a combination of ion and titanium sublimation pumps to a base pressure of the order of 10^{-10} torr ($\sim 10^{-8}$ Pa). The small signal counting rates encountered in the SAM and SDM modes required exposure times of 15–40 min.

The model biological surface consisted of a monolayer of poly- λ -methyl-glutamate (PMG) adsorbed onto a clean, polished stainless steel substrate. A pure carbon grid was subsequently vacuum deposited over the poly-amino acid layer. The sample was prepared as follows: The stainless steel sub-

strates, ~1 cm in diameter, were machined from 304 stainless steel and polished to optical flatness with alumina and diamond abrasives. The substrates were cleaned by rinsing in a sulphuric-chromic acid solution followed by a trichloroethylene, acetone, ethanol, and distilled water rinses. Poly- λ -methyl-glutamate¹² (0.9 mg) was dissolved in reagent grade chloroform (2 ml). A film of PMG cast onto distilled H₂O contained in a Pyrex trough was then deposited onto the stainless steel substrates by the Langmuir-Blodgett technique¹³. The PMG films were dried for 12 h at room temperature in a dust free environment. A carbon grid was vacuum deposited onto the PMG surface by shadowing the carbon flux with a 400 mesh electron microscope grid. The sample was rinsed in distilled water immediately before mounting it onto the specimen stage of the scanning Auger microprobe. The sample remained in the vacuum system for 1 h before analysis to allow the system pressure to fall to the 10⁻⁹ torr range.

Figure 1 shows an SEM view of C/PMG sample, and three different scanning Auger micrographs (SAM) of the same sample area. The relative elemental surface distribution of O, C and N is also shown. The C matrix is visible with high contrast in the standard SEM of the sample because of the large difference in secondary emission between C and the substrate. The C-SAM verifies the larger surface concentration of C at the matrix positions. The standard SEM gives no information of the presence of the PMG monolayer with this spatial resolution; only the topographical defects are shown on the underlying stainless steel substrate. The O-SAM shows attenuated oxygen signal from the FeO-Cr₂O₃ oxide layer on the stainless steel substrate¹⁴, whereas the N-SAM shows the PMG overlayer as a result of the surface amide concentration.

Single point Auger spectra which aided the interpretation of the SAMs are shown in Fig. 2 for the analysis beam centred over a pure carbon area (a), a PMG coated area (b), and cleaned substrate area (c) of the sample. The Auger spectra in the pure C region shows only C (at 272 eV) and a small O (510 eV) peak. The small relative surface concentration of O (<20 atomic %) after saturation of the sample with H₂O shows the essential hydrophobic nature of pure C at room temperature. The fact that the underlying high energy Fe Auger transitions (at 598, 651, 703 eV) are not visible indicated that the C film thickness is >50 Å.

Comparing the point Auger spectra from the PMG area with the cleaned substrate reveals the attenuation of the Fe and O transition by the PMG overlayer. In particular, the low energy Fe transition at 47 eV, for which escaping electrons have an attenuation length¹⁵ of ~5 Å, is completely attenuated by the PMG film. Assuming a simple model for the attenuation of low energy electrons:

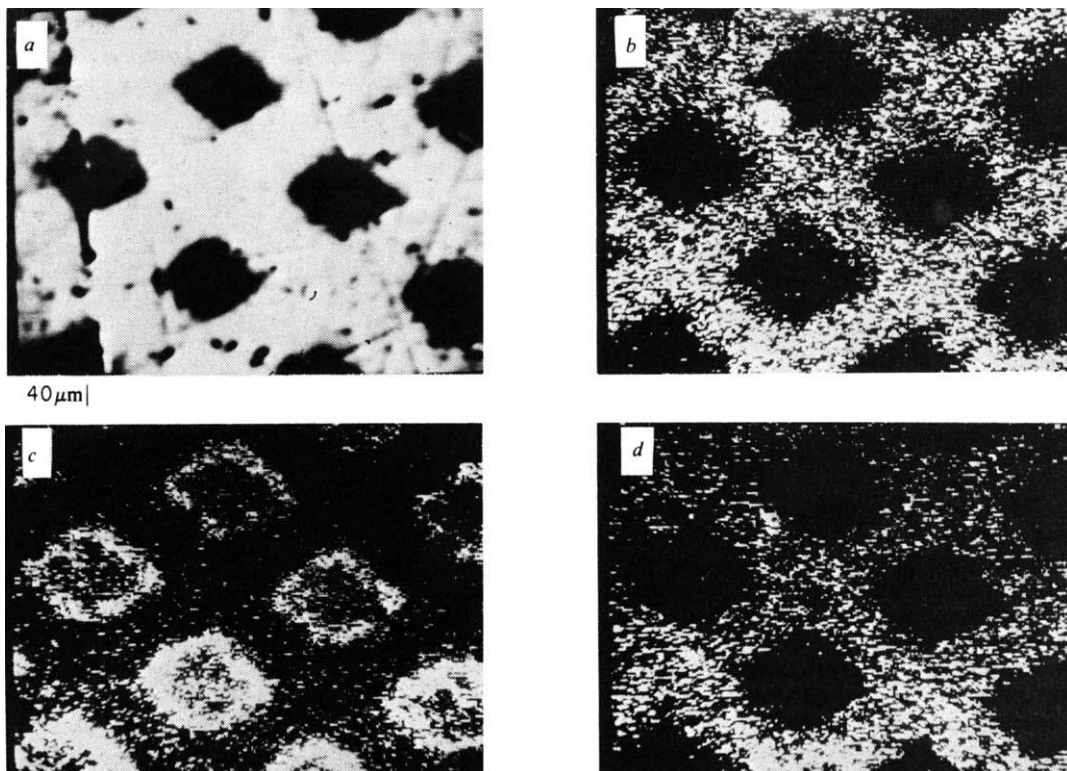
$$I/I_0 = \exp(-\lambda/d \sin \theta) \quad (1)$$

where λ = the inelastic attenuation length, d , the film thickness, and θ , the collection angle, the PMG film thickness is estimated to be of the order of 10–20 Å. Some fraction of the O Auger signal observed on the PMG is due to O in the PMG film, but this is difficult to estimate because of the presence of O in the stainless steel substrate. However, N is not present in significant quantities in the substrate, and we conclude that all the observed N in the PMG spectra and the N-SAM, is from PMG film. A rough calculation of the N concentrations based on relative sensitivity factors¹⁶ gives ~10 atomic % compared with the expected 16 atomic % for pure PMG. Little electron radiation damage was noted in the form of changes in the Auger spectra for the beam energy (2 keV), currents (75 nA), and exposure times (100–300 s) necessary for producing the micrographs.

Figure 3 shows a scanning desorption micrograph (SDM) of the C/PMG sample imaged with desorbing H⁺ ions. For comparison with the sample topography, an adsorbed current micrograph of the same area is also shown in Fig. 3. The desorbing H⁺ ions are seen only over the PMG area and not over the pure C areas. The detected H⁺ desorption signal was ~300 H⁺ ions per second or ~75 H⁺ ions per raster grid point. The shot noise in such a small signal accounts for the spotty appearance of the SDM.

The source of H⁺ is assumed to be ESD of adsorbed H₂O on the PMG although electron impact decomposition of the PMG layer may also contribute to the signal. The boundary between the PMG monolayer and adsorbed molecules is probably not distinct, and incident low energy electrons can interact with molecular groups in both phases. H⁺ can originate from adsorbed H₂O or CH₃ ligands; similarly OH⁺ or O⁺ can be desorbed from adsorbed H₂O or OH ligands. No ESD of OH⁺ or O⁺ ions was detected from the PMG sample. Its absence is consistent with the small size of the detected H⁺ signal. Studies of ESD of

Fig. 1 Scanning electron micrographs of test sample consisting of mechanically polished 304 stainless steel, on which a monolayer of poly- λ -methyl-glutamate (PMG) was adsorbed. A rectangular array of carbon was deposited over the unmasked portion of the PMG by evaporation of carbon through a 400 mesh stainless steel grid. *a*, Standard scanning electron micrograph (SEM) of the sample taken with a 2 keV primary beam. The remaining images are scanning Auger micrographs taken with: *b*, O(510 eV); *c*, C(272 eV); and *d*, N(381 eV) Auger electron emission. The SAMs were taken with 2 keV, 75 nA, primary beam, which scanned 250 lines per frame at a frame time of 187 s for C and O and 1,250 s for N. Magnification $\times 400$.



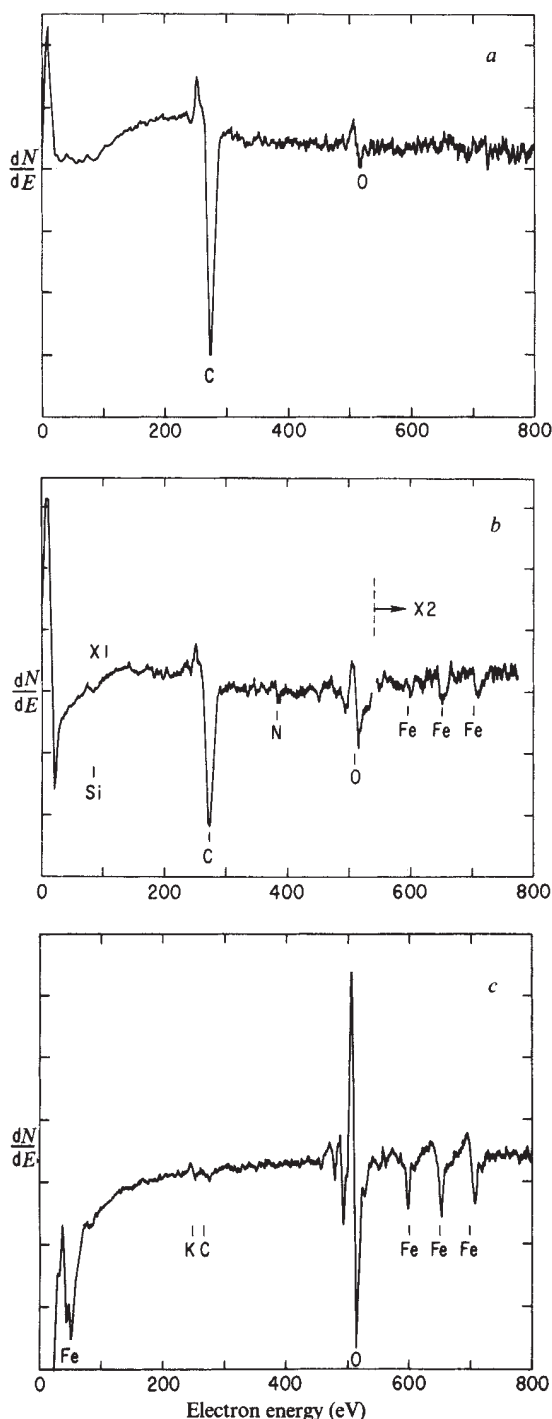


Fig. 2 Auger spectra of the C/PMG sample and substrate: spectra obtained when the primary beam is centred on: *a*, pure carbon area; *b*, PMG coated area of the sample; *c*, spectra obtained from the cleaned and degreased stainless steel substrate used for deposition of the C/PMG overlayer.

adsorbed H_2O on other surfaces^{1,6} usually show H^+ signals at least an order of magnitude larger than OH^+ or O^+ desorption signals.

To check the sample preparation procedure, the sample was removed from the microprobe and the PMG overlayer was dissolved in CHCl_3 for inspection by mass spectrometry. The parent peak (at 147 AMU), and the expected fragments of the PMG monomer were clearly visible in the mass spectrum as the solution was heated to 70 °C.

The difference in the ESD of H^+ from a hydrophobic form of carbon (pure carbon) and a hydrophilic form of carbon (a

proteinaceous hydrocarbon, PMG) has been used to image a matrix sample of the two materials exposed to H_2O . The relatively small desorption signal for this system (H^+ count rate of $\sim 300 \text{ s}^{-1}$) could be improved by incorporating an ion lens designed for high efficiency collection of ESD ions. The ion lens for this study was designed for SIMS for which overall signal count rates are rarely a constraint, but a small focusing area (and a small solid angle of acceptance) is the usual primary consideration¹⁷. The estimated ion collection efficiency for the ion lens system in this study is $\sim 10^{-3}$. This could be improved to $\sim 10^{-1}$ if the design constraints for SIMS were removed and the collection efficiency maximized. Any improvement in ion collection efficiency would allow shorter scan times per micrograph, and multiple adsorption-desorption cycles could be used in conjunction with the image storage and averaging to improve the signal-to-noise ratio.

The signal-to-noise ratio for SDM depends on the electron beam spot diameter; current density, J ; scan time, Δt ; ESD ionic cross-section, Q^+ and total cross-section, Q ; and ion collection efficiency, ϵ . The dependence of the signal-to-noise ratio on the lateral resolution (d) can be easily calculated, assuming the primary source of noise will be the shot noise in the comparatively small detected particle count (S) during an interval Δt . For desorbed ion signals, the signal-to-noise ratio will be

$$S/N = S/S^{1/2} = \left(\epsilon \frac{Q^+}{Q} n_0 \right)^{1/2} d \left(1 - \exp(-\Delta t/\tau) \right)^{1/2} \quad (2)$$

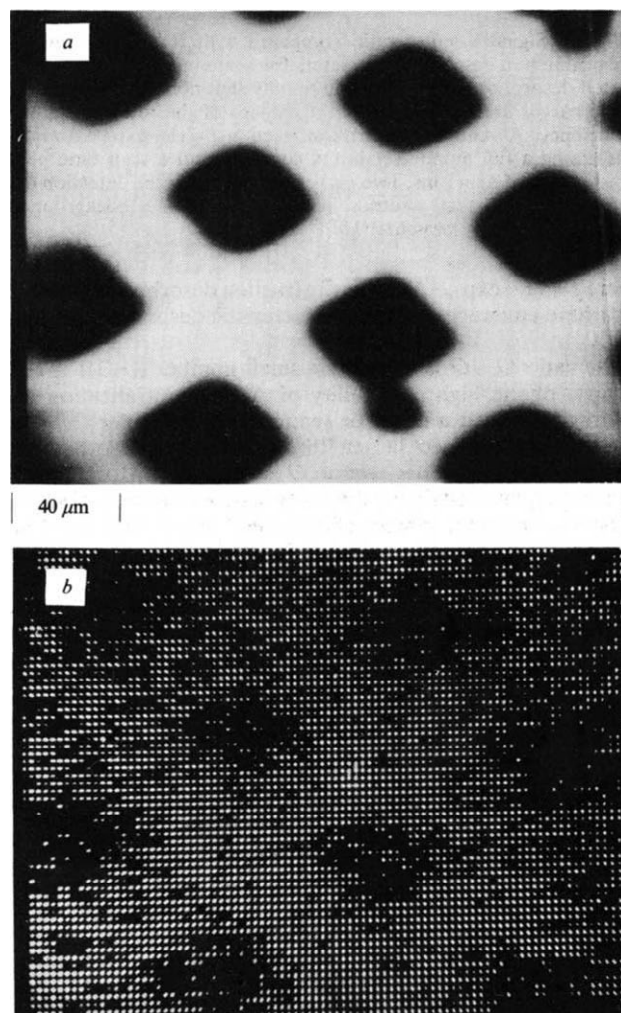


Fig. 3 *a*, Absorbed current micrograph of a neighbouring area of the sample shown in Fig. 1. *b*, Scanning desorption micrograph (SDM) of the same area imaged with desorbing H^+ ions. The beam voltage was 2.0 keV, beam current 1.7 μA , with 100 lines per frame scanned in 2,500 s. Magnification $\times 400$.

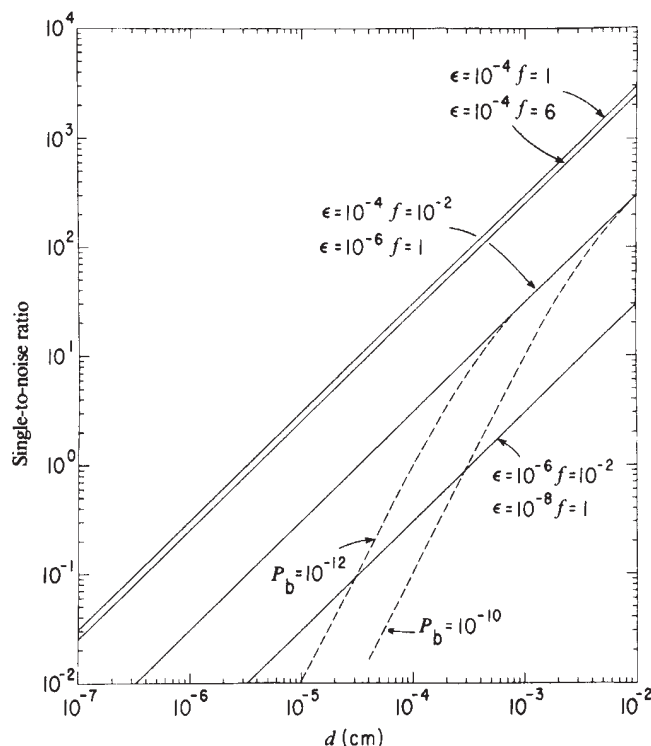


Fig. 4 Signal-to-noise ratio compared with resolution, where d = minimum resolvable diameter, for scanning electron-stimulated desorption microscopy where only shot noise is considered. The curves are plotted for various values of the total detection efficiency, ϵ , and the desorption fraction $f = (1 - \exp(-\Delta t/\tau))$, assuming a full monolayer initial coverage and a scan time per distance d , of $\Delta t = 2$ ms. Two curves are shown for the detection of desorbed (28 AMU) neutrals in the presence of a background pressure (10^{-10} – 10^{-12} torr).

where $f = (1 - \exp(-\Delta t/\tau))$ is the fraction desorbed of the initial adsorbate coverage n_0 . The characteristic desorption time τ is e/JQ .

The ratio Q^+/Q is typically a small number¹, $\sim 10^{-2}$ – 10^{-4} , because of the high probability of Auger neutralization of a desorbed ion near a metal or semiconductor surface¹⁸. Therefore, one might expect larger ESD signals from desorbed neutrals where the full cross-section Q is applicable. However, this gain is compensated by the difficulty in detecting neutral species (mass spectrometer ionizer efficiencies¹⁹ are at best 10^{-2}) and the additional noise source due to ionization of background gas molecules.

With this latter contribution the resulting signal-to-noise ratio for desorbed neutral signals is

$$\frac{\epsilon n_0 d^2 (1 - \exp(-\Delta t/\tau))}{[\epsilon n_0 d^2 (1 - \exp(-\Delta t/\tau)) + B P_b \Delta t]^{1/2}} \quad (3)$$

where P_b is the background partial pressure of the molecule of interest and B is a constant related to the ionizer efficiency. Equations (2) and (3) are plotted in Fig. 4 for several values of the detection efficiency, ϵ , and desorption fraction, f . The straight lines in Fig. 4 correspond to desorbed ion signals or to neutral signals where the background pressure of the desorption species is negligible.

For instrumental parameters for the microprobe used in this study¹⁰ and for ESD ionic cross-sections of 10^{-19} cm² for the adsorbate, the calculated spatial resolution limit is 2×10^{-4} cm for scanning desorption micrographs of minimal quality (signal-to-noise ratio is ~ 5).

3. Rork, G. & Consoliver, R. F. *Surface Sci.* **10**, 291–294 (1968).
4. Lichtman, D. & Campuzano, J. *J. appl. Phys. Suppl.* **2**, 189–192 (1974).
5. Dylla, H. F. & King, J. G. *Bull. Am. phys. Soc.* **21**, 241 (1976).
6. Joshi, A. & Davis, L. E. *J. Vac. Sci. Technol.* **14**, 1310–1313 (1977).
7. Poppa, H. & Bauer, E. *Surface Sci.* **97**, L309–L312 (1980).
8. Weaver, J. C. & King, J. G. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2781–2784 (1973).
9. DeMee, P. B. Frederickson, R. G. & Pope, R. S. in *Proc. 1977 Conf. on Scanning Electron Microscopy* Vol. II, 83–92 (IIT Research Institute, Chicago, 1977).
10. MacDonald, N. C., Hovland, C. T. & Gerlach, R. L. in *Proc. 1977 Conf. on Scanning Electron Microscopy*, Vol. I, 201–210 (IIT Research Institute, Chicago, 1977).
11. Gerlach, R. L. & Davis, L. E. *J. Vac. Sci. Technol.* **14**, 339–342 (1977).
12. Loeb, G. I. & Baier, R. E. *J. Colloid. Interface Sci.* **27**, 38–45 (1968).
13. Blodgett, K. B. *J. Am. chem. Soc.* **57**, 1007–1022 (1935).
14. Mathewson, A. G. in *Proc. int. Symp. on Plasma-Wall Interactions* 517–536 (Pergamon, Oxford, 1977).
15. Powell, C. J. *Surface Sci.* **44**, 29–46 (1974).
16. Davis, L. E. MacDonald, N. C. Palmberg, P. W. Riach, G. E. & Weber, R. E. *Handbook of Auger Electron Spectroscopy* (Perkin-Elmer, Eden Prairie, 1976).
17. Dawson, P. H. *Int. J. Mass. Spectrom. Ion Phys.* **17**, 447–467 (1975).
18. Hagstrum, H. D. *Phys. Rev.* **119**, 940–952 (1960).
19. Weiss, R. *Rev. Scient. Instrum.* **32**, 397–401 (1961).

Comparison of sulphide deposits from the East Pacific Rise and Cyprus

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Submarine sulphide deposits of the East Pacific Rise, discovered in 1978, at the mouth of the Gulf of California are compared here with fossil massive sulphide ore bodies. Two main types of massive sulphide ore bodies are defined in relation to the associated volcanism. The Cyprus ore shows striking analogies with East Pacific Rise deposits. The comparison is based on mineralogical observations and textural facies evidence. These similarities suggest that the genetic processes were identical.

Cu–Zn–Pb–Ag–Au polymetallic massive sulphide deposits associated with submarine volcanics have formed throughout geological time: from the Archaean (North-west Canada) up to the Tertiary (Miocene Kuroko deposits of Japan). These deposits are often rich and may reach 10% (or more) in metal contents. Massive or layered ore is generally underlain by a stockwork of sulphides associated with a hydrothermal alteration pipe. Hydrothermal exhalative circulations on the ocean floor which are contemporaneous with the volcanism are, in fact,

Table 1 Mineralogical association

Deposit minerals	CYAMEX	ALVIN	Cyprus
Magnetite		T	
Haematite		T	
Pyrite	A	A to F	A
Marcasite	F	R	F to R
Pyrrhotite	T	R	T
Chalcopyrrhotite	F to R	F to R	
Chalcopyrite	F to R	F to R	F to R
Wurtzite or sphalerite	F	A	R
Bornite		F	R
Digenite	T	R	R
Chalcocite		T	R
Idaite		T	T
Covellite	T	T	T
Galena		T	T
Jordanite		T	T
Tennantite		T	T
Native Gold			T
Native Sulphur		R	
Goethite	A	T	T
Anhydrite	T	A	
Baryte	T	R	
Quartz			R
Opal	R	R	R
Carbonates		T	R

A, Abundant; F, frequent; R, rare; T, trace.

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1. Madey T. E. & Yates J. T. *J. Vac. Sci. Technol.* **8**, 525–555 (1971).
2. Menzel, D. *Surface Sci.* **47**, 370–383 (1975).

the most characteristic common features of these deposits. Two types of massive sulphide deposits can be distinguished. The first shows a close association with calc-alkaline acid volcanics being part of acidic to basic differentiation¹. The Archaean deposits in Canada, the Palaeozoic Huelva Province in Spain, and the Miocene Kuroko deposits of Japan are all of this type. The second type of copper rich mineralization exhibited by the Cyprus deposits, is associated only with basic tholeiitic volcanism and has less economic importance.

The two types have very different tectonic environments which explains the different nature of the volcanism involved. The first type is found in Island Arcs related to subduction zones (for example, the Kuroko deposits of Japan) and the second type is located in ophiolite complexes inferred to be the products of an accreting plate boundary (Mid-Ocean-Rise). Sedimentary features such as compositional banding are characteristic of the first type while collomorph textures² are typical of the second. The latter shows more important structural controls.

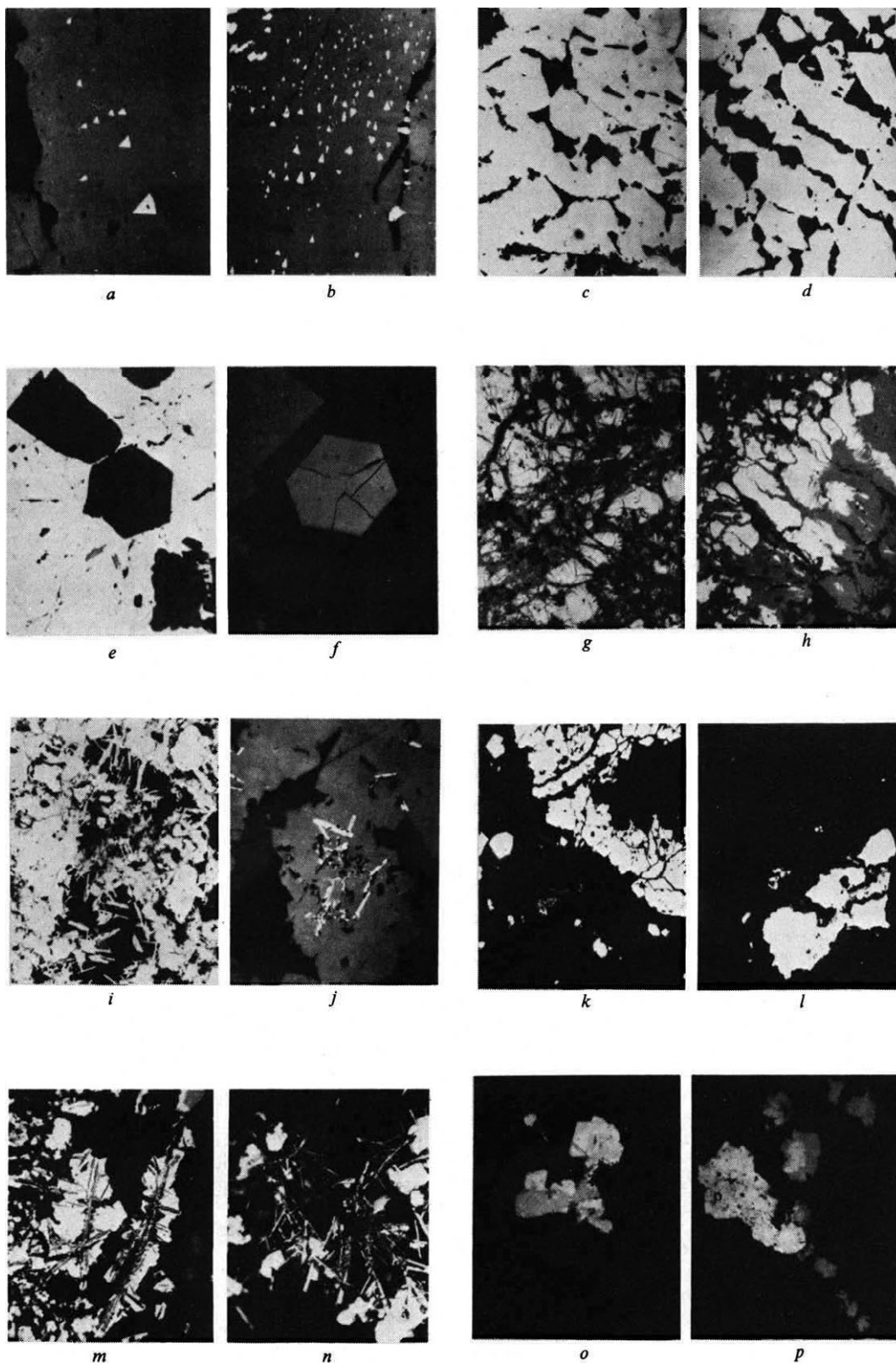


Fig. 1 *a*, triangular-shaped inclusions of chalcopyrite in wurtzite from Cyprus. Polished section, PPL $\times 200$. *b*, Similar triangular-shaped inclusions of chalcopyrite in ALVIN wurtzite. Polished section, PPL $\times 160$. *c*, Massive chalcopyrite formed by aggregates of euhedral grains. The interstitial spaces are open, from Cyprus. Polished section, PPL $\times 150$. *d*, Similar massive chalcopyrite from ALVIN. Polished section, PPL $\times 150$. *e*, Hexagonal crystal of ZnS (originally in wurtzite) included in pyrite from Cyprus. Polished section, PPL $\times 500$, in oil immersion. *f*, hexagonal wurtzite in ALVIN sample (included in araldite). Polished section, PPL $\times 160$. *g*, Replacement of chalcopyrite by bornite, from Cyprus. Polished section, PPL $\times 150$. *h*, Similar textures in ALVIN sample. Polished section, PPL $\times 150$. *i*, Lamellar chalcopyrite on massive chalcopyrite (pyrite is whiter and harder) from Cyprus. Polished section, PPL $\times 150$. *j*, Lamellar chalcopyrite included in the zinc sulphide (medium-grey) of ALVIN. Polished section, PPL $\times 600$ oil immersion. *k*, Pyrite rimmed by opal, from Cyprus. Polished section, PPL $\times 150$. *l*, Pyrite rimmed by opal, from ALVIN. Polished section, PPL $\times 160$. *m*, Lamellar bornite embedded in pyrite, from Cyprus. Polished section, PPL $\times 150$. *n*, Lamellar pyrrhotite partially altered and associated with some pyrite, from ALVIN. Polished section, PPL $\times 600$ oil immersion. *o*, Jordanite (grey) associated with galena (white), from Cyprus. Polished section, PPL $\times 950$ oil immersion. *p*, Jordanite (grey) surrounding galena (white), pyrite (Py), from ALVIN. Polished section, PPL $\times 950$ oil immersion.

In Cyprus the mineralization occurs at the top of an ophiolitic series. It is situated in and above the lower pillow-lavas (LPL) underlying the upper pillow-lavas (UPL) which have the manganiferous umbers at the top. The pyritic ore forms a stockwork which becomes massive towards the top and is enriched in copper (1–10%) and locally in zinc. This massive mineralization is usually concentrated in basin-like depressions at the top of the LPL and is often coated by 'ochres' which are thought to be a submarine gossan and equivalent to submarine actual metalliferous sediments. Nearly all the deposits are at the intersection of important synsedimentary faults which have been reactivated after the ore deposition.

Relatively recent hydrothermal sulphide deposits were discovered in 1978 during the CYAMEX expedition in the mouth of the Gulf of California. They are situated at a depth of 2,600 m on the accreting plate boundary of the East Pacific Rise^{6,7}. The sulphides occur in 'mounds' which are 10 m high and average 5 m in diameter. These chimneys are situated ~700–800 m away from the ridge axis and occur in small depressions (~30 m in width and depth). A later expedition with the American submersible ALVIN discovered active hydrothermal vents^{8–10} creating massive sulphide mounds very close to the ridge axis. These samples are fresher and usually better crystallized than the CYAMEX ones. Sampling and their relative proximity to the rift axis might be partly responsible for the difference in textures and paragenesis. Abundant goethite and rare gypsum are characteristic of the CYAMEX samples which represent a submarine gossan.

Previous mineralogical studies of deep-sea sulphide deposits from the CYAMEX area^{11,12} revealed that the samples were markedly altered. However, no comparison could be made with ore deposits associated with ophiolitic complexes such as Cyprus. New data from the study of unaltered samples from Alvin as well as new samples from Cyprus suggest that the genetic processes were similar for both recent (ALVIN)^{13–16} and fossil deposits (Cyprus). This analogy can be substantiated from: (1) textural evidence and facies; and (2) similarities of mineral paragenesis and more specifically, the presence or absence of some trace minerals.

(1) Textural evidence and facies: neither deposit shows banding. In Cyprus the ore shows various facies: brecciated, disseminated, in stockwork and massive. In ALVIN the ore is massive and sometimes brecciated while in CYAMEX (altered). Collomorphic and arborescent (dendritic) crystalline growth can be observed in both cases although such textures are much more abundant in CYAMEX–ALVIN. In both, massive chalcopyrite is formed by the aggregation of euhedral grains. The interstitial spaces are open (Fig. 1c, d). Chalcopyrite also forms triangular inclusions in zinc sulphide which could represent an epitaxial growth on the wurtzite crystals, which are present in both deposits (Fig. 1a, b).

One can also observe: well developed hexagonal crystals of wurtzite in CYAMEX–ALVIN and probable sphalerite pseudomorphs after wurtzite in Cyprus ore (Fig. 1e, f). Wurtzite spherulites have now been identified in Cyprus ore. They are absent in ALVIN samples but common in CYAMEX where they crystallize *in situ* from hexagonal wurtzite. In both ALVIN and Cyprus, various mineral species (such as chalcopyrite, and bornite show lamellar structure which could represent a replacement of former platy minerals (Fig. 1i, j, m, n). Chalcopyrite is often replaced by bornite in both deposits (Fig. 1g, h). Bornite or pyrite are often coated by a thin rim of sphalerite. Pyrite, chalcopyrite or zinc sulphide are often coated by opal and sometimes rimmed by carbonates and are observed in Alvin and Cyprus deposits (Fig. 1k, l).

(2) Paragenetic similarities: Jordanite ($\text{Pb}_5\text{As}_2\text{S}_8$) which is usually found in very different settings (such as karst deposits, and deposits in the sedimentary cover) is a relatively rare mineral; however, it occurs in both mineral assemblages (Fig. 1o, p).

Trace minerals such as tellurides and cassiterite common in and characteristic of many volcanogenic¹⁷ deposits are unknown

in Cyprus and CYAMEX–ALVIN. However, these trace minerals seem to occur more often in Island Arc massive sulphide ore. Furthermore—the existence of finely expressed cassiterite seem to be related to the presence of acidic volcanism.

Finally some differences must also be considered. Chalcopyrrhotite^{18,19} (a high temperature form of cubic cubanite) is completely absent in Cyprus. This can be understood if one considers that chalcopyrrhotite is an unstable phase which was preserved in CYAMEX–ALVIN samples as a result of quenching. Therefore chalcopyrrhotite is thought to be easily altered by diagenetic or low-grade metamorphic processes. Wurtzite, which is abundant in CYAMEX–ALVIN and rare in Cyprus, has a similar behaviour pattern probably reverting to sphalerite in fossil deposits. Pyrrhotite is abundant in ALVIN but scarce in CYAMEX where it changes to marcasite and iron sulphates. In Cyprus it is rare or altered to marcasite. As noted above, ALVIN is situated very near the rift axis whereas CYAMEX is 1 km away and can be considered as a fossil deposit in which the easily altered pyrrhotite has nearly all been transformed. Cyprus distance⁶ to the fossil rift axis is 2.5 km.

The mechanism of formation of a hydrothermal ore deposit on the ocean floor has been described elsewhere^{20–23} before the discovery of ALVIN hydrothermally active vents. Direct observation of the eruption of hot hydrothermal fluids on the ocean floor has provided important new data. From stable^{24,25} and radiogenic isotope^{26,27} measurements we know that seawater pervades the permeable oceanic basalts and circulation occurs as a result of the elevated temperatures associated with nearby magma chamber(s).

The efficiency with which hot water dissolves metals in basalts has been demonstrated elsewhere^{28–32}. Partial chemical analyses^{10,33} show that the hydrothermal fluids are enriched in calcium, lithium, silicon and manganese and depleted in magnesium. As predicted by Spooner²², a localized sulphide precipitation occurs on the ocean floor as a result of a quenching phenomenon: when 350 °C (maximum) hydrothermal fluids erupts into the +2 °C ocean bottom water. The stockwork mineralization underlying the massive ore would represent the channel through which the hydrothermal fluids had ascended. Equivalent sulphide veins could be formed within the upper few hundred metres of the basaltic crust in ALVIN zone, as a result of adiabatic expansion of the hydrothermal fluids³³. The ochre and umbers at the top of Cyprus ore bodies can be compared with recent metalliferous sediments found near submarine volcanic or rift zones in the Pacific³⁴.

The mineralogical assemblages and textures of ALVIN samples are directly related to their genetic process of formation. Low-grade metamorphic ore from Cyprus shows very striking analogies which suggest a similar genetic process. The differences in mineralogy are readily understandable when one considers the alteration and diagenetic processes. These results confirm the distinctions based on tectonic environment and ore mineralogy between the two types of massive sulphide deposits. (1) Mineralizations associated with calc-alkaline volcanics, where trace minerals such as cassiterite and tellurides (mainly hessite and altaite) are frequent. (2) Mineralizations associated with basic tholeiitic volcanism where cassiterite is never observed and tellurides are absent, as in Cyprus and CYAMEX–ALVIN.

However, deposits with frequent tellurides³⁵ are present in ophiolite series but are restricted to the basic to ultrabasic lower unit³⁶. The tellurides observed in such deposits are hessite and melonite (a nickel telluride which indicates a basic influence). Lead telluride (altaite) is absent and clausthalite (a lead selenide) is formed instead.

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1. Pouit, G. *Mém. Soc. géol. Fr.* 115–126 (1976).
2. Adamides, N. G. *Trans. Instn Min. Metal.* **84**, B17–B23, 317–322 (1975).
3. Constantinou, G. & Covett, G. J. S. *Trans. Instn Min. Metal.* **81**, B34–B46, 334–345 (1971).
4. Constantinou, G. *Econ. Geol.* **68**, 83–86 (1973).

5. Hutchinson, R. W. & Searle, D. F. *Soc. Miner. Geol. Jap. Spec. Iss.* **3**, 198–205 (1971).
6. CYAMEX Scientific team *EOS* **59**, 1198 (1978); *C.r. hebdo. Acad. Séanc. Sci., Paris* **287** (1978).
7. Francheteau, J. et al. *Nature* **277**, 523–528 (1979).
8. MacDonald, K. C. et al. *Earth planet. Sci. Lett.* **48**, 17 (1980).
9. Edmond, J. M. et al. *EOS* **60**, 864 (1979).
10. Spiess, F. N. et al. *Science* **207**, 1421–1433 (1980).
11. Hekinian, et al. *Science* **207**, 1433–1444 (1980).
12. Picot, P. et al. *Docum. Bur. Rech. Geol. Min.* No. 20 (1980).
13. Oudin, E. *Docum. Bur. Rech. Geol. Min.* (in the press).
14. Haymon, R. et al. *EOS* **60**, 864 (1979).
15. Haymon, R. M. & Kastner, M. *Descriptive Catalog of Rise Sulphide Samples from the East Pacific Rise*, 21°N, 510 (Reference Series 79–28, 1979).
16. Haymon, R. et al. *Earth planet. Sci. Lett.* (in the press).
17. Aye, F. et al. *Bull. Miner.* **101**, 139–147 (1978).
18. Cabri, J. L. et al. *Can. Miner.* **12**, 33–38 (1973).
19. Cabri, J. L. *Econ. Geol.* **68**, 443–454 (1973).
20. Ohmoto, H. et al. *Econ. Geol.* **69**, 947–953 (1974).
21. Bonatti, E. A. *Rev. Earth planet. Sci. Lett.* **3**, 401–431 (1975).
22. Spooner, E. T. C. et al. *Contr. Miner. Petrol.* **42**, 287–304 (1973).
23. Spooner, E. T. C. *Trans. Instn Min. Metal.* 58–68 (1976).
24. Heaton, T. H. E. et al. *Trans. Instn Min. Metal.* 42–57 (1976).
25. Sheppard, S. M. F. *Trans. Instn Min. Metal.* 25–41 (1976).
26. Spooner, E. T. C. et al. *Geochim. cosmochim. Acta* **41**, 873–890 (1977).
27. Chapman, H. J. et al. *Earth planet. Sci. Lett.* **35**, 71–78 (1978).
28. Bischoff, J. L. et al. *Earth planet. Sci. Lett.* **25**, 385–397 (1975).
29. Hajash, A. *Contr. Miner. Petrol.* **53**, 205–226 (1975).
30. Bischoff, J. L. et al. *Proc. 2nd int. Water-Rock Interaction Symp.* Strasbourg (1977).
31. Seyfried, W. et al. *Earth planet. Sci. Lett.* **34**, 71–77 (1977).
32. Mottl, et al. *Geochim. cosmochim. Acta* **43**, 869–884 (1978).
33. Bischoff, J. L. *Science* **207**, 1465–1469 (1980).
34. Guillemot, D. et al. *Proc. Int. Ophiolite Symp.*, Cyprus (1980).
35. Bouladon, J. et al. *Bull. Bur. Rech. Geol. Min.* No. 1, 24–41 (1968).
36. Constantinou, G. *Proc. int. Ophiolite Symp.*, Cyprus (1980).

Are high rates of sulphate reduction associated with anaerobic oxidation of methane?

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Classical models of sulphur diagenesis in marine sediments are based on the assumption that the rate of sulphate reduction is first order with respect to oxidizable particulate organic carbon (POC). This assumption requires that oxidizable POC, sulphate concentration and the sulphate reduction rate be highest at the top of the sulphate reduction zone and decrease exponentially with increasing sediment depth^{1,2}. However, to explain recent observations of concave upwards methane distributions, the anaerobic consumption of methane has been proposed. Furthermore, it has been proposed that this consumption takes place near the bottom of the sulphate reducing zone where sulphate concentrations are low^{3–6}. Thus, if sulphate reducing bacteria are associated with the anaerobic oxidation of methane, a peak in sulphate reduction rate might be expected in this deep consumption zone⁷. The importance of the process in sedimentary sulphur diagenesis is indicated by calculations estimating that 30–75% of the downward sulphate flux at depth may be consumed by methane oxidation within this zone^{6,8,9}. We present here profiles of sulphate reduction rate in anoxic sediments that show distinct local maxima at the depth where the anaerobic oxidation of methane would be expected. Our measurements were made during July and August 1978 in Saanich Inlet, an anoxic fjord located on the south-east side of Vancouver Island, British Columbia. The inlet has a shallow sill (~70 m) which restricts circulation of the deeper water (maximum depth 225 m) inside the basin to the extent that for about 8 months of the year the bottom waters contain no oxygen, nitrate or nitrite, and significant concentrations of free H₂S are present¹⁰. As the deep waters contain hydrogen sulphide, the inlet is an ideal location for studying sedimentary sulphate reduction because reactions with oxygen and the effects of burrowing organisms can be neglected.

All sediment samples were taken at a station located in 225 m of water using a hydraulically damped multiple corer that avoids disturbance of the sediment water interface¹¹. Typical distributions of SO₄²⁻ and total dissolved sulphide reflect the strongly reducing sedimentary environment in Saanich Inlet (Fig. 1a). Sulphate was exhausted at depths of ~20–30 cm in the sediment column and sulphide concentrations increased rapidly with depth, reaching maximum concentrations near the depth of sulphate depletion. Below this depth dissolved sulphide concentrations decreased. This type of dissolved sulphide profile is characteristic of strongly reducing sediments and is due to sulphide production in the zone where sulphate is present and removal as FeS below¹². Sediment porosity was high at the surface, ~0.96 cm³ per cm³ and decreases only slightly in the upper 40 cm of the sediment column (Fig. 1b). At any given depth sulphate reduction rates were somewhat variable; however, the main features of the profiles were consistent from core to core. In general, the highest rates were observed at the surface and decreased with increasing depth until ~10 cm. Below this depth a local maximum was observed in all but one core (Fig. 2).

The profiles presented here cannot be modelled within the framework of current diagenetic models. Assuming steady state and no absorption of sulphate by sediment particles, the distribution of sulphate or any other soluble species can be described by equation (1)¹:

$$\frac{\partial}{\partial z} \left(\phi D \frac{\partial [\text{SO}_4]}{\partial z} \right) - \frac{\partial}{\partial z} (\phi \omega [\text{SO}_4]) + R = \frac{\partial [\text{SO}_4]}{\partial t} = 0 \quad (1)$$

where ϕ is the porosity, ω is the sedimentation rate, z is depth taken positively downward, t is time, D is the apparent diffusion coefficient of the sulphate in sediment, and R is the sulphate reduction rate. It is generally assumed that the sulphate reduction rate is first order with respect to oxidizable POC. Thus, because POC is nondiffusible, we can also write

$$-\omega \frac{\partial G}{\partial z} = kG = -\frac{R}{\alpha} \quad (2)$$

where G is the concentration of metabolizable POC, α is a stoichiometric coefficient equal to the number of sulphate ions reduced per atom of carbon oxidized and k is a first-order rate constant¹. Assuming constant values of ω , α and k , the solution to equation (2) with the boundary conditions that $G = G_0$ at

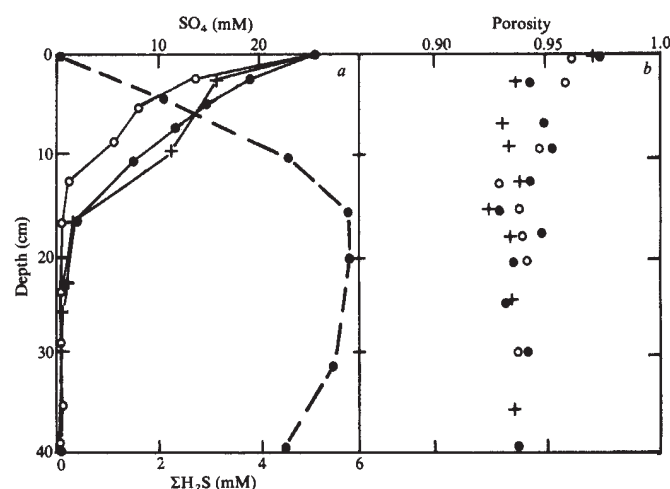
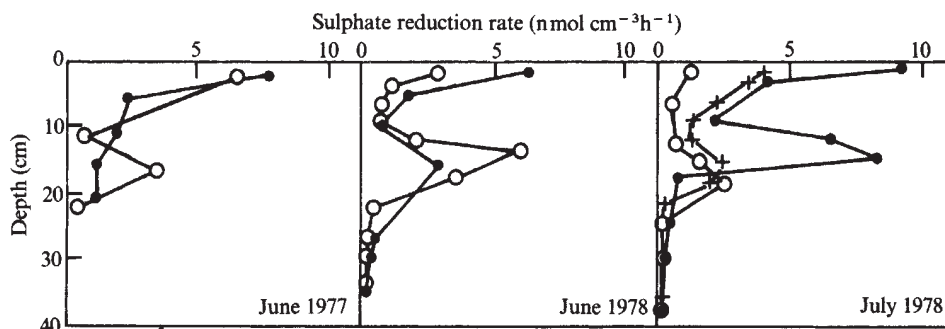


Fig. 1 Typical profiles of sulphate (solid lines, a), total dissolved sulphide (dashed line, a) and porosity (b). For sulphate and total dissolved sulphide, cores were sectioned and the pore water expressed into nitrogen filled test tubes using a gas (N₂) operated squeezer. Dissolved sulphide analyses¹⁴ were performed immediately, while sulphate samples were frozen for later analyses¹⁵. Bulk sediment porosity was determined by weight loss after drying at 80 °C and corrected for salt content. Symbols distinguish measurements made on replicate cores.

Fig. 2 Profiles of sulphate reduction rate. Sulphate reduction rates were determined on whole sediments using the method of Jørgensen¹⁶ as modified by Nedwell and Abram¹⁷. Symbols distinguish measurements made on replicate cores.



$z = 0$ and G approaches zero as z approaches infinity is $G = G_0 \exp[-(k/\omega)z]$. This solution requires that the concentration of metabolizable POC and therefore the sulphate reduction (αkG) decrease exponentially with sediment depth. This is clearly not the case for the sulphate reduction rate data from Saanich Inlet.

That the sulphate reduction rate does not decrease exponentially as predicted by the model indicates that there is either a corresponding increase in oxidizable POC at ~ 15 cm, or that other, presumably dissolved, substrates are being utilized. Although there is no method available to determine metabolizable POC, total POC has been determined at our sampling station⁹ and no subsurface peak is evident. In fact, the total POC data can be fitted reasonably well with an exponentially decreasing oxidation rate function (Fig. 3b).

To investigate the possibility that dissolved organic carbon (DOC) was the substrate responsible for the subbottom peak in the sulphate reduction rate, we also measured the DOC

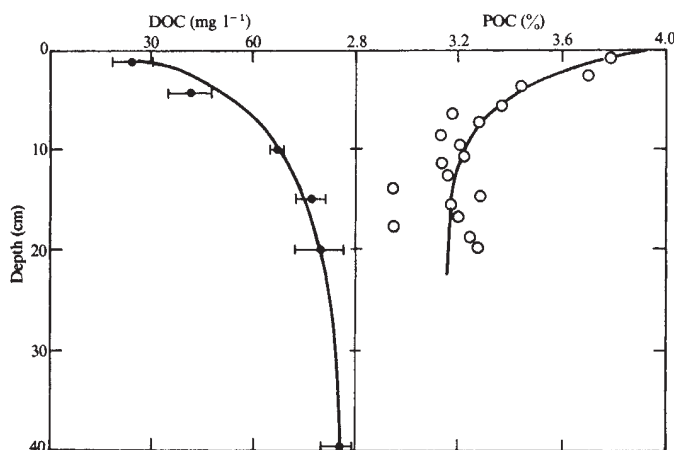


Fig. 3 Dissolved organic carbon (DOC) and total particulate organic carbon (POC) in Saanich Inlet sediments. DOC was determined by wet oxidation¹³. Samples were acidified, and bubbled with nitrogen to remove dissolved inorganic carbon. Consequently, volatile components such as CH_4 were also removed. The total POC profile is redrawn from ref. 9⁹. Both the DOC and total POC profiles were modelled using equation (1). For the general case we substitute C for $[\text{SO}_4]$ in equation (1), where C represents either DOC or total POC. The best fit to both the DOC and total POC data was obtained by assuming an exponential rate term. ($P = P_0 \exp[-\beta z]$, where β is a decay coefficient and P is substituted for R in equation (1). For DOC, P represents a net production rate which decreases exponentially from a surface value of P_0 . For POC, P represents an oxidation which decreases exponentially from a surface value of P_0). Given the boundary conditions, $C = C_0$ at $z = 0$ and $C \rightarrow C_\infty$ as $z \rightarrow \infty$ (where C_∞ is finite), and assuming the values of ω and D presented by Murray *et al.*⁹, the solution to the general form of equation (1) with an exponential rate term is $C = (C_0 - C_\infty) \exp[-\beta z] + C_\infty$, where $(C_0 - C_\infty) = P_0 / (D\beta^2 + \omega\beta)$ (ref. 15). The model fits are shown as solid lines.

concentration at various depths within the sediments (Fig. 3a). However, because of the analytical method used, our DOC measurements do not include volatile organic components such as CH_4 (ref. 13). DOC concentration increased steadily with depth and showed no apparent consumption in the region of the sulphate reduction peak. The DOC distribution is best modelled if we assume an exponentially decreasing net production rate.

Thus, both the total POC and nonvolatile DOC profiles can be modelled as smooth functions of depth, but, within the context of classical diagenetic models it is impossible to explain the subsurface peak in sulphate reduction rate because the models predict an exponentially decreasing rate function. However, the peak in the sulphate reduction rate profile is located in the depth zone where sulphate concentrations approach zero. This is exactly the depth zone where the anaerobic oxidation of methane, which would not be included in our DOC measurement, has been proposed to take place. Investigations in other marine sediments have shown that a significant fraction of the downward sulphate flux at depth is consumed within the anaerobic methane oxidation zone^{6,8}. That the magnitude of anaerobic methane oxidation is sufficient to support the sulphate reduction peak observed in Saanich Inlet is indicated by a previous study in which an areal methane oxidation rate of $7.7 \times 10^{-12} \text{ mol cm}^{-2} \text{ s}^{-1}$ was calculated⁹. Assuming oxidation takes place through the reaction, $\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HS}^- + \text{HCO}_3^- + \text{H}_2\text{O}$, and that the reaction occurs principally in a 10 cm thick depth zone where sulphate concentrations approach zero, this areal methane oxidation rate would be equivalent to a volumetric sulphate reduction rate of $2.8 \text{ nmol cm}^{-3} \text{ h}^{-1}$. This value is in good agreement with the observed data (Fig. 2). We therefore conclude that the high rates of sulphate reduction we observe at depth are associated with the anaerobic oxidation of methane, either directly or indirectly, through reactions coupled to other substrates.

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- Berner, R. A. in *The Sea*, Vol. 5 (ed. Goldberg, E. D.) 427-450 (Wiley-Interscience, New York, 1974); *Early Diagenesis* (Princeton University Press, New Jersey, 1980).
- Lerman, A. in *The Sea* Vol. 6 (ed. Goldberg, E. D.) 695-738 (Wiley-Interscience, New York, 1977).
- Barnes, R. D. & Goldberg, E. D. *Geology* **4**, 297-300 (1976).
- Reeburgh, W. S. & Heggie, D. T. *Limnol. Oceanogr.* **22**, 1-9 (1977).
- Martins, C. S. & Berner, R. A. *Limnol. Oceanogr.* **22**, 10-25 (1977).
- Reeburgh, W. S. *Earth planet. Sci. Lett.* **47**, 345-352 (1980).
- Mountfort, D. O., Asher, R. A., Mays, E. R. & Tiedje, J. M. *Appl. Envir. Microbiol.* **39**, 686-694 (1980).
- Reeburgh, W. S. in *The Dynamic Environment of the Ocean Floor* (eds Fanning, K. A. & Manheim, F. T.) (Heath, Boston, in the press).
- Murray, J. W., Grundmanis, V. & Smethie, W. M. *Jr. Geochim. cosmochim. Acta* **42**, 1101-1026 (1976).
- Anderson, J. J. & Devol, A. H. *Estuar. Coast. mar. Sci.* **1**, 1-10 (1973).
- Pamatmat, M. M. *Limnol. Oceanogr.* **16**, 536-550 (1971).
- Goldhaber, M. B. & Kaplan, I. R. *Soil Sci.* **119**, 42-55 (1975).
- Menzel, D. W. & Vaccaro, R. F. *Limnol. Oceanogr.* **9**, 139-142 (1964).
- Cline, J. D. *Limnol. Oceanogr.* **14**, 454-458 (1969).
- Jørgensen, B. B. *Geomicrobiology J.* **1**, 11-27 (1978); 29-47 (1978).
- Macchi, G. R., Cescon, B. S. & Mameli-D'Errico, D. *Arch. Oceanogr. Limnol.* **16**, 163-171 (1969).
- Nedwell, D. B. & Abram, J. W. *Estuar. Coast. mar. Sci.* **6**, 341-351 (1978).

Albumin systematics of the extinct mammoth and Tasmanian wolf

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Knowledge of the molecular systematics of living species¹⁻³ has provided a framework, independent of morphology, for evaluating the genetic relationships of living forms. Although amino acids have been found in many animal and plant fossils⁴, genetic information has generally not been obtained from the small amounts of surviving, chemically degraded protein. However, Westbroek *et al.*⁵ have described immunological reactions to material from 60-Myr-old molluscs, and Lowenstein^{6,7} has reported the identification by radioimmunoassay (RIA) of species-specific collagen and serum factors in primate and bovine fossils. We report here the use of RIA to detect and characterize albumin in the soft tissues of two recently extinct species, the Siberian mammoth (*Mammuthus primigenius*) and the Tasmanian wolf (*Thylacynus cynocephalus*). Mammoth albumin was found to be very similar to, and immunologically equidistant from, the albumins of the two living species of elephants, Indian (*Elephas maximus*) and African (*Loxodonta africana*). Tasmanian wolf albumin stands in the same relationship to those of the two Australian marsupial dasyurids, *Dasyurus* and *Dasyuroides*.

Our RIA is a double-antibody radioimmunometric assay with polyvinyl microtitre plates as the solid-phase ligand^{6,8}. Fossil extracts, or protein standards, are left for 1 h in the microtitre cups, where some protein binds irreversibly to the plastic. The rest is washed out with a solution containing 2% chicken egg protein to block any further binding of plastic to protein. Next, rabbit antisera against albumins⁹ are added to the cups and left at room temperature for 24 h; some antibody binds to the plastic-bound albumin, the rest is washed out. About 1 ng ¹²⁵I-labelled goat anti-rabbit γ -globulin (GARGG) is then added to each cup and left for 24 h. The amount of GARGG bound increases with the amount of rabbit antibody present.

Table 1 Direct RIA reactions of mammoth and other albumins

Antigen	% Uptake of radioactive second antibody			
	IE	AE	Mn	H
Mammoth (<i>Mammuthus primigenius</i>)	10.0	10.3	0.25	0.20
Indian elephant (IE) (<i>Elephas maximus</i>)	10.0	10.3	0.84	3.2
African elephant (AE) (<i>Loxodonta africana</i>)	9.7	10.0	0.83	3.1
Manatee (Mn) (<i>Trichechus manatus</i>)	2.1	2.2	10.0	2.8
Human (H) (<i>Homo sapiens</i>)	2.1	2.2	0.80	10.0

With rabbit anti-albumin concentration fixed at 10^{-4} M, homologous albumin concentration in the plastic cup was adjusted to give 10% uptake of labelled GARGG. Mammoth extract was adjusted to give 10% uptake with anti-IE albumin. The mammoth extract reacts far more specifically with antisera to elephant albumin than to the others. Reciprocal values are not particularly good with this method, and the cross-reactions fail to show the relationship (albeit distant) of the manatee with the elephants. The two elephant albumins react identically.

Table 2 Immunological similarities and distances among mammoth, elephant and other albumins

Antigen	Inhibition		Mn	B	H
	IE	AE			
Mammoth	0.94	0.92	0.30	0.05	0.03
Indian elephant (IE)	1.0	0.92	0.33	0.10	0.07
African elephant (AE)	0.94	1.0	0.45	0.10	0.07
Manatee (Mn)	0.39	0.40	1.0	0.06	0.12
Bison (B)	0.33	0.33	0.20	1.0	0.12
Human (H)	0.22	0.22	0.23	0.01	1.0

For all albumins but mammoth, inhibition was achieved by premixing inhibitor serum (10^{-2} M) with antiserum (10^{-4} M) homologous to the solid-phase albumin (10^{-5} M). Thus inhibitor exceeded bound antigen by a factor of 10^3 . 1.0 indicates complete inhibition (no uptake of radioactive antibody) and immunological identity; 0 would represent no inhibition and no immunological similarity. For the mammoth, where the amount of albumin available was insufficient for use as an inhibitor and clean antisera to it were not available¹³, the values were obtained by comparing the ability of its plastic-bound albumin to bind the various antisera, inhibited and uninhibited, with that shown by the other albumins. For example, AE albumin inhibits the reaction between mammoth albumin and anti-IE albumin no more or less strongly than that between that antiserum and IE albumin. Thus mammoth albumin is as distant from that of AE as is IE albumin. The reciprocal reaction, using IE albumin as the inhibitor for anti-AE, tells you that mammoth albumin is also as distant from IE albumin as is that of AE. The same logic follows for all the other comparisons. Reciprocal values are fairly good, decreasing in reliability as immunological similarity decreases. The two elephant species are clearly differentiated in this approach, so that we were able to identify seven out of seven different 'unknown' sera, four Indian and three African. The relation of the manatee to the elephants is also apparent.

The unbound radioactive solution is washed out, the plate cut up and individual cups assayed for radioactivity in a scintillation counter. Final radioactivity is a function of both the amount of albumin on the cup and the specificity of the first antiserum for that albumin. Sensitivity of the method (smallest concentration of albumin detectable) is ~ 1 ng ml⁻¹—about one-thousandth the amount detectable with the sensitive microcomplement fixation (MCF) technique which has been used extensively for studying albumin evolution^{3,9-12}. In the 'direct' RIA described here, specificity (discrimination among closely related species) is, however, inferior to that of MCF or quantitative precipitin analysis.

RIA specificity can be greatly improved by having an albumin in solution in the cup competing with the solid-phase albumin for available antibody. This then becomes an inhibition or competitive binding technique⁸. Soluble rabbit anti-albumin is premixed with excess albumin or serum (100–1,000 times the concentration originally applied to the plastic cup), and binds essentially all the antibody directed against common determinants of the two competing albumins, permitting only those antibody populations which react more strongly with the determinants on the plastic-bound albumins to bind to the latter. The more similar the two competing albumins, the less will be the uptake of antibody (and hence radioactivity) on the plastic.

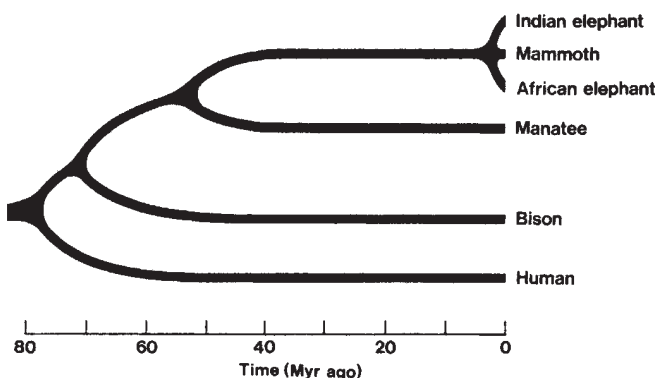


Fig. 1 An albumin phylogeny of the mammoth and related forms, based on the data of Tables 1 and 2 and ref. 13. Those data suggest relatively similar amounts of albumin evolution along the various lineages represented and thus we feel justified in adding the approximate time scale included. We assume a divergence time of 80 Myr between the ungulate and non-ungulate placentals.

Table 3 Direct RIA reactions of Tasmanian wolf and other marsupial albumins

Antigen	% Uptake of radioactive second antibody				
	D	Dd	My	E	Be
Tasmanian wolf (<i>Thylacinus</i>)	9.1	10.0	8.3	6.8	4.0
<i>Dasyurus</i> (D)	10.0	8.5	8.3	4.8	3.5
<i>Dasyuroides</i> (Dd)	10.9	10.0	8.9	6.0	4.3
<i>Myrmecobius</i> (My)	7.7	5.2	10.0	3.7	2.0
<i>Echymipera</i> (E)	5.8	5.5	4.9	10.0	3.8
<i>Bettongia</i> (Be)	3.5	2.6	3.4	4.9	10.0
<i>Caluromys</i>	2.5	1.1	1.5	1.1	0.4
Human	2.1	0.6	0.9	0.7	0.3

The direct reactions of Tasmanian wolf muscle extract with various marsupial anti-albumins show that it groups with the dasyurids, and among those is slightly closer to *Dasyurus* and *Dasyuroides* than to *Myrmecobius*. In order of increasing distance are the peramelid *Echymipera* and the macropodid *Bettongia*, both Australian genera. Further still are the South American woolly opossum *Caluromys* and the placental mammals represented by human serum. As described in Table 1 legend, antiserum concentration was fixed at 10^{-4} M and serum concentration adjusted to give homologous uptake of 10%. Even with the direct reaction, reciprocity is good enough so that branching orders are evident from inspection.

We used this method to distinguish the albumins of the African and Indian elephant, which are indistinguishable by MCF, and to characterize the similarities of the fossil albumins with albumin from related species. Each determination of antibody binding was run in duplicate or triplicate, with about 5% variation from run to run. Each study of the fossil albumins was run several times, with essentially identical results. Although only a small proportion of the GARGG added may be bound, especially in inhibited reactions, the amount remains statistically above that of the 'blank', consisting of cups coated with chicken egg protein. With the direct RIA method, maximum binding of antibody is generally achieved with antiserum concentration about 10^{-3} M; but maximal inhibition occurs at lower concentrations, usually 10^{-4} M. To calibrate the RIA results, we compared them with a standard method for determining evolutionary distances—MCF—which has been tested on hundreds of albumins. With various albumin immunological distances, we found a consistent correlation between the results of the two methods; we therefore felt confident in applying this calibration to the fossil proteins.

The 40,000-yr-old baby Siberian mammoth known as Dima was discovered near Magadan, USSR in 1977. A specimen of right thigh muscle was obtained, ground in buffer and tested for albumin by MCF, immunodiffusion and RIA. A portion was also injected into rabbits to make antisera¹³. Initially, neither MCF nor immunodiffusion detected mammoth albumin directly, but after its presence had been shown by RIA [at $\sim 1 \mu\text{ml}^{-1}$ (or $\sim 1\%$ the amount extractable from fresh muscle)], it was also demonstrated, after further concentration, by the other methods, and was shown to be very similar to elephant albumin¹³. Antisera raised against the mammoth muscle suspension gave the strongest reactions with elephant albumin¹³.

Direct RIA mammoth reactions are given in Table 1. Neither direct RIA, MCF, nor immunodiffusion distinguishes between the albumins of the Indian and the African elephant. To determine whether mammoth albumin is immunologically closer to one or the other of these, we first tested seven different elephant sera as 'unknowns' and identified correctly the four Indian and three African sera. Next we compared the mammoth to these two species and found its albumin immunologically equidistant from them, giving a 93% cross-reaction with each using the inhibition technique described above (Table 2). The inhibition procedure apparently exaggerates the small difference between the two elephant albumins; judging by MCF; or quantitative precipitin analysis they are >99% identical. The next most

similar albumin, that of the manatee (*Trichechus*), gives only a 40% cross-reaction, while *Bison* and *Homo* albumins are at 22% and 15%, respectively. These data and their MCF homologues facilitate the construction of an albumin phylogeny (Fig. 1) which corresponds well to current ideas about the relationships of these species¹⁴. The molecular distance between the two elephants when assessed by RIA inhibition, MCF comparisons of albumin and transferrins, and electrophoretic comparisons of the serum proteins, is, however, only about half that for the corresponding proteins in humans, chimpanzees and gorillas. The divergence time of the latter trio, on the biochemical evidence, is ~ 5 Myr (refs 10, 11) which implies a shorter divergence time for the elephants, contrary to prevailing opinion¹⁴.

While no one, to our knowledge, has suggested that the mammoth is not closely related to living elephants, the affinity of the recently extinct Tasmanian wolf with the dasyurids, the Australian marsupial carnivores with which it is generally grouped, has been seriously questioned. The marked morphological convergence of this species on dogs and foxes, its corresponding departure from any other Australian form, and strong cranial, dental and skeletal resemblances to the extinct South American borhyaenids have suggested¹⁵⁻¹⁷ affinities to that group rather than the dasyurids.

We obtained three museum specimens of thylacine material: dried muscle (0.4 g) adhering to a bone collected before 1893 in Tasmania and two untanned skin samples of similar weight collected early in the twentieth century. Each specimen was finely ground in a 0.2 M EDTA buffer, pH 7.4, and extracted for 48 h. Extracts were tested by RIA with antisera to the albumins of *Dasyurus* (tiger cat), *Dasyuroides* (kowari), *Myrmecobius*

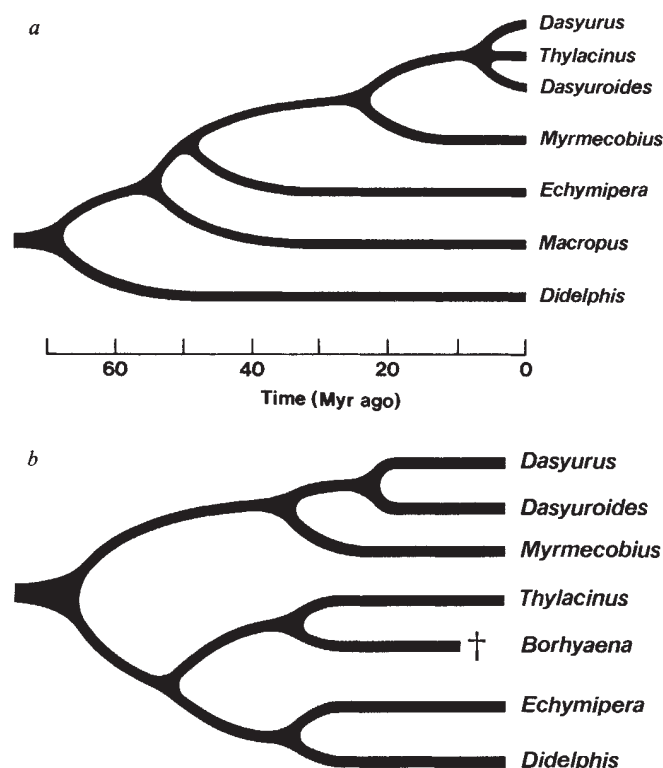


Fig. 2 a, An albumin phylogeny of the marsupial carnivores and related forms, based on the RIA data in Tables 3 and 4 and on ref. 21. All Australian marsupial lineages other than dasyurids and peramelids probably stem from the lineage shown terminating in *Macropus*. All South American marsupials other than caenolestids stem from the line labelled *Didelphis*. b, Marsupial carnivore phylogeny based on Archer's¹⁸ dental phenogram. No time scale is implied. Note the markedly different placements of the *Thylacinus* and *Echymipera* lineages in this scheme, compared with that in a. *Borhyaena* is a long-extinct form not yet subject to biochemical study.

Table 4 Immunological similarities and distances of Tasmanian wolf and related marsupials

	T	D	Inhibition Dd	My	E
<i>Thylacinus</i> (T)		0.86	0.82	0.67	0.30
<i>Dasyurus</i> (D)	7		0.84	0.59	0.28
<i>Dasyuroides</i> (Dd)	9	8 (11)		0.71	0.32
<i>Myrmecobius</i> (My)	17	23 (41)	15 (39)		0.29
<i>Echymipera</i> (E)	52	55 (94)	49 (76)	54 (89)	

Averages of reciprocal inhibition similarities (RIA^{IS}) are given above the diagonal, except for *Thylacinus*, for which no anti-albumin was available. As with the mammoth, its similarities were obtained by inhibiting antisera to the albumins of the most closely related species, *Dasyurus* and *Dasyuroides*. Anti-D was inhibited with Dd serum and vice versa, giving 86% and 82% inhibition, respectively. The RIA^{ID} (inhibition difference) values (below the diagonal) are calculated from the RIA^{IS} values (above the diagonal) according to the equation $ID = -100 \log_{10} IS$. The corresponding MCF values are given in parentheses. Clearly, the RIA^{ID} values here are slightly more than half the corresponding MCF values and a wider range of comparisons suggests that $MCF^{ID} = 1.7 RIA^{ID}$. Thus, the RIA^{ID} values approximate the divergence times in Myr between the pairs of species involved.

(numbat), *Echymipera* (bandicoot) and (*bettongia* (rat kangaroo or bettong). The first three are dasyurids, the fourth a peramelid and the last a macropodid. The sera had been prepared for a project previously reported²¹. The three thylacine extracts gave identical species-specific cross-reactions, reacting as though they contained ~1% of the albumin that would be found in extracts of fresh tissues. This result is very similar to that obtained from the mammoth muscle and suggests that a small fraction of the albumin in soft tissues is sequestered and protected from leaching and denaturants. After extraction, the insoluble muscle tissue could also bind antibodies to marsupial albumins, indicating the presence of immunologically active albumin in this residue. We were able to extract 10 times more albumin from muscle than from skin and used this material for detailed comparisons.

Antisera to *Dasyurus* and *Dasyuroides* albumins reacted maximally with the thylacine extract (Table 3), indicating close immunological similarity. Progressively weaker reactions were seen with antisera to *Myrmecobius*, *Echymipera* and *Bettongia* albumins. These relationships were confirmed and quantified by inhibition reactions (Table 4), which showed *Dasyurus*, *Dasyuroides* and *Thylacinus* to be immunologically equidistant, the others more distant. The other largish dasyurid, the Tasmanian devil (*Sarcophilus*), gives a MCF albumin distance to *Dasyurus* of only six units, about half that given by *Dasyuroides* and, by implication, *Thylacinus*. We therefore suggest that the Tasmanian wolf is part of a quite recent dasyurine radiation and separated from the lineages leading to *Dasyurus*-*Sarcophilus* and *Dasyuroides* in the late Miocene some 6–10 Myr ago (Fig. 2). The positions of *Thylacinus* and *Echymipera* differ profoundly in our scheme (Fig. 2a) and that of Archer (Fig. 2b).

These results should help settle the controversy about thylacine affinities. We agree with Simpson¹⁹ and Marshall²⁰ that thylacine resemblances to borhyaenids must be due to parallel or convergent evolution, for the thylacine albumin is that of a very recently derived dasyurid. This may be the first of many phylogenetic issues to be affected by the protein immunology of extinct forms.

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1. Ayala, F. J. (ed.) *Molecular Evolution* (Sinauer, Sunderland, Massachusetts, 1976).
2. Dayhoff, M. O. *Atlas of Protein Sequence and Structure* Vol. 5, Suppl. 3, (National Biomedical Research Foundation, Washington DC, 1978).
3. Wilson, A. C., Carlson, S. S. & White, T. J. *A. Rev. Biochem.* **46**, 573–639.
4. Wyckoff, R. W. G. *The Biochemistry of Animal Fossils* (Scientific, Bristol, 1972).
5. Westbroek, P. H. et al. *Paleobiology* **5**, 151–167 (1979).
6. Lowenstein, J. M. in *Biogeochemistry of Amino Acids* (ed. Hare, P. E.) 41–52 (Wiley, New York, 1980).
7. Lowenstein, J. M. *Naturwissenschaften* **67**, 343–346 (1980).
8. Tsu, T. T. & Herzenberg, L. A. in *Selected Methods in Cellular Immunology* (eds Mishell, B. B. & Shiigi, S. M.) Ch. 18 (Freeman, San Francisco, 1980).
9. Sarich, V. M. *Syst. Zool.* **18**, 286–295 (1969).
10. Sarich, V. M. & Cronin, J. E. in *Molecular Anthropology* (eds Goodman, M. & Tashian, R. E.) 141–170 (Plenum, New York, 1976).
11. Sarich, V. M. *Nature* **265**, 24–28 (1977).
12. Sarich, V. M. *Nature* **245**, 218–220 (1973).
13. Prager, E. M., Wilson, A. C., Lowenstein, J. M. & Sarich, V. M. *Science* **209**, 287–289 (1980).
14. Coppens, Y., Maglio, V. J., Madden, C. T. & Beden, M. in *Evolution of African Mammals* (eds Maglio, V. J. & Cooke, H. B. S.) 336–367 (Harvard University Press, 1978).
15. Bensch, B. A. *Trans. Linn. Soc. London (Zool.)* **9**, 83–217 (1903).
16. Sinclair, W. J. *Rep. Princeton Univ. exped. Patagonia* **4**, 333–460 (1906).
17. Archer, M. J. *Linn. Soc.* **59**(3), 217–322 (1976).
18. Archer, M. *Aust. J. Zool. Suppl. Ser.* **39**, 1–34 (1976).
19. Simpson, G. G. *Am. Mus. Novit.* **1118**, 1–6 (1941).
20. Marshall, L. G. *Syst. Zool.* **26**, 410–425 (1977).
21. Maxson, L. R., Sarich, V. M. & Wilson, A. C. *Nature* **255**, 397–400 (1975).

Intense exercise, bone structure and blood calcium levels in vertebrates

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Sudden, large-scale fluctuations of systemic calcium concentrations are believed to be harmful to vertebrates. Consequently, it is generally accepted that vertebrates relatively closely monitor and regulate the calcium levels in blood and other tissues¹, often by endocrinological mechanisms which act over long time periods to promote calcium deposition on or dissolution from bone^{2,3}. However, after intense bouts of maximal activity, virtually all vertebrates experience a significant reduction in blood pH as a result of concomitant increases in blood P_{CO_2} and lactate concentrations^{4,5}, and this might be expected to have wide-ranging physiological effects. We now present evidence that (1) blood plasma calcium concentrations rise abruptly and significantly as a result of intense muscular activity in all vertebrates investigated which have osseous skeletons, but not those with cartilaginous skeletons, and (2) the source of the excess calcium is bone.

We examined changes in plasma calcium concentrations and blood pH following bouts of intense activity in a variety of vertebrates of differing skeletal composition. Animals were stimulated to maximal activity until exhausted (~5 min) by chasing them by hand. Blood samples were taken from these individuals 10 min after cessation of activity. Similar determinations were also made on resting individuals. The results of these experiments (Table 1) indicate a significant rise in blood plasma calcium concentration after activity in all species with a bony skeleton ($P < 0.03$, t -test of each species) but no significant elevation in species with a cartilaginous skeleton (lamprey, dogfish; $P > 0.05$, t -test). Similar proportional increments in calcium concentration were also noted in whole blood samples taken before and after identical activity regimes in *Salmo*, *Squalus* and *Rattus*.

A separate set of experiments was conducted on the lizard *Iguana iguana* to determine the time course of activity-induced changes in blood pH, lactate and calcium concentrations. Results of these experiments (Fig. 1) indicate that, in *Iguana*, (1) the rise in plasma calcium concentration after initiation of

activity is rapid and detectable within the first 5 min of sustainable activity, (2) the time course of elevations in plasma calcium concentration parallel temporal changes in blood pH and lactate concentration after initiation of activity, and (3) elevation of plasma calcium concentration as a result of intense activity may persist for 30 min or longer.

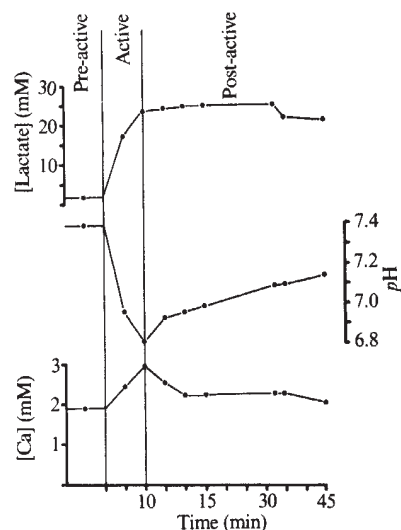


Fig. 1 Calcium and lactate concentrations and pH in samples of whole blood taken before, during and after 10 min of treadmill walking at 0.5 km h⁻¹ in a 0.6-kg *Iguana iguana*. Three adult iguanas were exercised on a motor-driven treadmill at 35 °C. The experimental apparatus and protocol have been described elsewhere¹¹. Blood was sampled before, during and after activity via an in-dwelling T-shaped cannula in the femoral artery. Anaerobically collected blood samples were analysed for pH as described in Table 1 legend. Additional whole blood samples were precipitated in 2× volume 0.6 M perchloric acid and analysed for [Ca] as described in Table 1 legend and [lactate] with a Boehringer-Mannheim enzymatic analysis kit¹². Iguanas were allowed to rest for 30–60 min before pre-active blood sampling and were then exercised for 10 min at 0.5–1.0 km h⁻¹; the lizards walked steadily during the first 5 min and became fatigued and required prodding during the next 5 min. They were then allowed to recover from activity for 30–60 min. All animals experienced a rise in whole blood calcium, ranging from 0.45 to 1.15 mM, during the first 5 min of activity. Blood calcium levels peaked at 10 min activity, increasing an average of 53% above pre-active values and remained elevated for at least 30 min after exertion. Blood pH declined and lactate concentrations increased precipitously during activity.

What is the source of this additional plasma calcium? Major reservoirs of calcium are located in bone, scales in fish, skeletal muscle and other large organs, such as the liver. To determine the role of scales in the rise of plasma calcium in osseous piscine species, plasma calcium levels before and after activity were measured in 'scaleless' trout. These animals had scales (except those associated with the lateral line system) removed by mechanical abrasion, but remained healthy. Following a 48-h recovery period, scaleless individuals were subjected to the experimentation described in Table 1 legend. Plasma calcium concentration rose by 68% following intense activity in scaleless individuals (resting: 2.42 ± 0.05 s.e. mM Ca, pH 7.52, $n = 5$; post-active: 3.99 ± 0.11 s.e. mM Ca, pH 7.17, $n = 7$) the magnitude of hypercalcaemia being almost identical ($P > 0.5$, t -test) to that observed after activity in normally scaled individuals (Table 1). Thus, fish scales do not seem to be the major source of calcium involved in the phenomena described here.

To investigate calcium release by soft tissues, we measured calcium concentrations of skeletal muscle and liver tissue before and after activity in rainbow trout and rattlesnakes. The results (Table 2) indicate that these tissues exhibit either static or slightly increased calcium concentrations following intense activity, rather than decreasing as would be anticipated. Thus, neither can easily be assumed to be the source of the increased plasma calcium. The lack of post-active hypercalcaemia in fish with cartilaginous skeletons implies that skeletal muscle and other soft tissues in these animals do not release significant quantities of calcium even during activity-generated acidosis. It is consequently unlikely that these tissues are the source of hypercalcaemia in vertebrates with osseous skeletons. Thus, bone seems to be the most likely source of this excess calcium.

The mechanism resulting in post-active hypercalcaemia might simply involve dissolution of a fraction of the crystalline calcium hydroxyapatite compartment of the osseous skeleton as a result of systemic lactic acid accumulation and pH depression. This hypothesis resembles that proposed for longer-term parathormone function, in which bone resorption is thought to occur, at least in part, by local secretion of lactic acid⁶. Also, the solubility of bone calcium hydroxyapatite *in vitro* has previously been shown to be sensitive to pH change⁷.

If increased acidity of tissue fluids associated with burst activity is indeed a causal factor in bone dissolution, our data have important implications for the evolution of the vertebrate skeleton. Recent evidence^{4,5} indicates that, throughout their history, vertebrates have been capable of high levels of 'burst' activity supported by anaerobic generation of lactic acid. Activity-associated dissolution of ossified skeletal tissues resul-

Table 1 Effect of intense activity on blood pH and plasma calcium concentration in a variety of vertebrates (mean $\pm$ s.e.)

Species	Resting			Post-active		
	pH	[Ca] mM	No.	pH	[Ca] mM	No.
Osseous skeleton						
Rainbow trout (<i>Salmo gairdneri</i>)	7.49 $\pm$.04	2.31 $\pm$.01	4	7.10 $\pm$.03	3.97 $\pm$.16	9
Longnose gar (<i>Lepisosteus osseus</i>)	7.77 $\pm$.04	2.56 $\pm$.07	4	7.03 $\pm$.05	4.14 $\pm$.05	5
White crappie (<i>Pomoxis annularis</i>)	7.93 $\pm$.04	2.43 $\pm$.03	4	7.42 $\pm$.05	2.82 $\pm$.12	6
Largemouth bass (<i>Micropterus salmoides</i>)	7.88 $\pm$.04	2.49 $\pm$.06	8	7.28 $\pm$.05	3.01 $\pm$.05	6
Rattlesnake (<i>Crotalus viridis</i>)	7.45 $\pm$.02	2.58 $\pm$.08	5	6.82 $\pm$.07	4.08 $\pm$.13	7
Laboratory rat (<i>Rattus rattus</i>)	7.50 $\pm$.01	2.61 $\pm$.05	4	7.33 $\pm$.02	3.02 $\pm$.10	6
Human (<i>Homo sapiens</i>)	7.42 $\pm$.01	2.65 $\pm$.02	6	7.19 $\pm$.02	2.88 $\pm$.04	6
Cartilaginous skeleton						
Pacific brook lamprey (<i>Lampetra pacifica</i>)	7.91 $\pm$.04	2.61 $\pm$.04	6	7.23 $\pm$.05	2.40 $\pm$.09	8
Pacific spiny dogfish (<i>Squalus suckleyi</i>)	7.92 $\pm$.05	4.31 $\pm$.07	4	7.35 $\pm$.02	4.54 $\pm$.11	4

To determine the resting blood pH, 75- μ l blood samples were taken anaerobically in heparinized syringes from previously undisturbed individuals of each species. Blood was collected from the ventral aorta of fish and from tail incisions in snakes and rats. Elapsed time between first handling and sample procurement was <15 s; struggling by animals was minimal during this period. The pH of the sample was measured immediately with a Radiometer-Copenhagen BMS 3 mark II blood microunit connected to a Radiometer-Copenhagen PHM 71 mark II acid-base analyser. The temperature of the electrode was regulated at the body temperature of the experimental subjects. Immediately after withdrawal of the previous blood sample, another sample of ~ 200 μ l was withdrawn from each individual in a non-heparinized syringe. Plasma from these samples was then diluted at a 1:12 ratio with a calcium-suspending solvent (containing 3.60×10^{-3} M La_2O_3 ; 5.0×10^{-2} M HCl) and then analysed for calcium concentration on a Jarrell-Ash atomic absorption spectrophotometer equipped with a laminar flow burner. Different individuals of each species were then stimulated to maximal activity by manual prodding until exhaustion occurred (within ~ 5 min). After a 10-min rest period, blood samples were collected and analysed for whole blood pH and plasma calcium concentration as described above. Each species was maintained and exercised at an environmentally realistic temperature ($T_b = 12$ °C, *Lampetra*, *Salmo* and *Squalus*; $T_b = 18$ °C, *Pomoxis*, *Lepisosteus* and *Micropterus*; $T_b = 35$ °C, *Crotalus*). Finger prick blood samples from humans were collected before and 10 min after running exercise to complete fatigue on a staircase (~ 4 min). Samples were analysed as above. No significant differences in blood [Ca] were noted between individuals of different sexes in any species.

Table 2 Calcium concentration in liver and skeletal muscle before and after activity ($n = 4$ rest, 4 post-active)

Species	Calcium concentration (mM $\pm$ s.e.)			
	Liver		Skeletal muscle	
	Rest	Post-active	Rest	Post-active
Rainbow trout (<i>Salmo gairdneri</i>)	0.15 $\pm$.02	0.18 $\pm$.03	0.31 $\pm$.05	0.40 $\pm$.05
Rattlesnake (<i>Crotalus viridis</i>)	0.32 $\pm$.03	0.33 $\pm$.05	0.37 $\pm$.01	0.39 $\pm$.03

Samples of liver and skeletal muscle tissue (epaxial muscle from *Salmo* and longissimus dorsi from *Crotalus*) were obtained from decapitated specimens that had been previously undisturbed or exercised to exhaustion and allowed to recover for 10 min. Samples were homogenized in a Sorvall Omnimixer, and analysed for calcium concentration by atomic absorption spectrophotometry as described in Table 1 legend.

ting in marked acute hypercalcaemia may have been a problem for vertebrates since their origin and we therefore suggest that there has been continuous selection for minimization of these effects. That selection may, at least in part, account for the evolution of several seemingly unrelated skeletal features in different groups of vertebrates. For example: (1) A primarily cartilaginous adult skeleton is acquired and maintained by several distantly related groups of vertebrates, including extant cyclostomes, elasmobranchs, chimaerans and primitive actinopterygians (Chondrostei). Although cartilage is often highly mineralized, it is virtually non-vascularized, so that the relative surface areas of cartilage exposed to post-active acidic tissue fluids must be orders of magnitude less than that for bone, which is highly vascularized. Thus, far lower rates of post-active mineral dissolution and hypercalcaemia are to be expected for cartilaginous species. (2) Acellular bone develops in extinct heterostracan ostracoderms and advanced teleost fish⁸. Acellular bone in modern teleosts is distinguished from cellular bone in other vertebrates by the absence of osteocytes and osteoclasts in the former. Consequently, like cartilage, acellular bone is considerably less vascularized than cellular bone and undoubtedly has a considerably reduced surface area exposed to post-active acidic tissue fluids. (3) An epithelia-like 'barrier' of osteocytes effectively isolates the bone-tissue fluid compartment from the tissue fluid continuum of the rest of the body. Such compartmentalization, well known in mammals⁹, might serve to isolate the hydroxyapatite of bone from sudden, activity-associated pH fluctuations experienced by the soft tissues following intense activity. Each of these various developments functions, at least in part, to isolate or 'protect' most of the calcium-laden skeletal components from body tissue fluids. They have resulted in a dramatic reduction in activity-related hypercalcaemia in animals with 'protected' skeletons compared with those with

apparently little or no isolation of osseous tissues from the rest of the body (Fig. 2).

Thus, activity may profoundly alter blood calcium levels, even in mammals and other vertebrates with 'protected' osseous skeletons. Investigators must handle experimental subjects carefully as over-excitement may result in a rise in increments of plasma calcium concentration similar to those described here. The physiological consequences of this naturally induced, short-term hypercalcaemia have yet to be examined. However, non-activity-related acute hypercalcaemia in mammals is known to induce many deleterious physiological responses, including bradycardia, sinus arrhythmia and hypertension, disruption of normal renal function, nausea, confusion and disorientation¹⁰. Investigation of similar effects in vertebrates experiencing activity-related hypercalcaemia seems warranted.

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- Phang, J. M. & Weiss, I. in *Handbook of Physiology*, Sect. 7, Vol. 7 (ed. Aurbach, G.) 157-168 (American Physiological Society, Washington DC, 1976).
- Bentley, P. *Comparative Vertebrate Endocrinology* (Cambridge University Press, London, 1976).
- Turner, C. & Bagnara, J. *General Endocrinology* (Saunders, Philadelphia, 1971).
- Bennett, A. F. A. *Rev. Physiol.* **40**, 447-469 (1978).
- Ruben, J. A. & Bennett, A. F. *Nature* **286**, 886-888 (1980).
- Neuman, W. F., Neuman, M. W. & Meyers, C. W. *Am. J. Physiol.* **236**, 244-248 (1979).
- McLean, F. C. & Urist, M. R. *Bone* (University of Chicago Press, 1968).
- Hancox, N. M. *Biology of Bone* (Cambridge University Press, London, 1972).
- Talmage, R. V. *Clin. Orthopaed.* **67**, 210-224 (1969).
- Parfitt, A. M. & Kleerekoper, M. in *Clinical Disorders of Fluid and Electrolyte Metabolism* (eds Maxwell, M. & Kleeman, C.) 947-1151 (McGraw-Hill, San Francisco, 1980).
- Gleeson, T. T., Mitchell, G. S. & Bennett, A. F. *Am. J. Physiol.* **239**, R174 (1980).
- Bennett, A. F. & Licht, P. *J. comp. Physiol.* **81**, 277 (1972).

Somatic and behavioural postnatal effects of fetal injections of nerve growth factor antibodies in the rat

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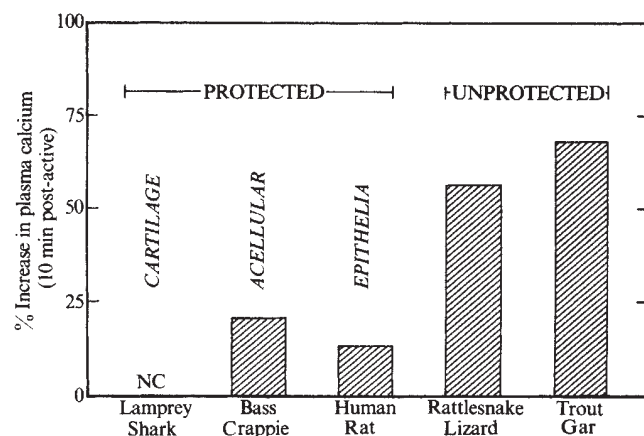


Fig. 2 Percentage increase in plasma calcium concentration after activity in vertebrates of differing skeletal type.

Previous work has shown that injections to neonatal mammals of a specific antiserum to the nerve growth factor (AS-NGF) produce irreversible destruction of sympathetic ganglia^{1,2}. The animals used in these studies were mice and rats. Because this treatment does not damage other nerve cells or produce adverse effects in other tissues and organs, the immunosympathectomized young and adult rodents do not exhibit—at gross inspection—somatic or behavioural differences compared with controls except for a marked ptosis³. Differences become apparent, however, when the treated rodents are submitted to physiological, pharmacological and behavioural tests⁴. We report here the dramatic effects that are produced by injections of purified antibodies to NGF in rat fetuses. A preliminary account of this work has already been presented^{5,6}, and similar effects have been demonstrated in another laboratory.

2.5 NGF was purified by the method of Bocchini and Angeletti⁷. After two successive carboxymethyl cellulose chromatographies, NGF was run in SDS-polyacrylamide gel electrophoresis and the corresponding band cut out, eluted from the gel matrix, mixed with complete Freund's adjuvant and injected into rabbit to produce antibodies. These were purified

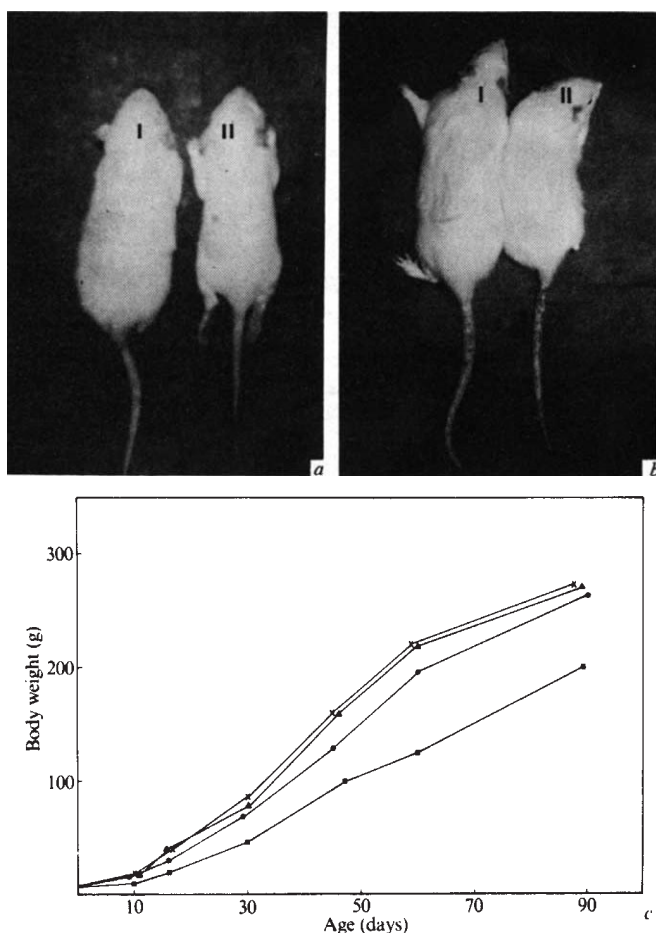


Fig. 1 Control (I) and experimental (II) rats killed respectively at 3 weeks (a) and 2 months (b) of age. Control rats were injected pre- and postnatally with 0.15 M NaCl for their first 3 weeks. Experimental rats were injected with 40 μ g of purified NGF antibodies dissolved in 0.15 M NaCl. Further details are included in the text. c, Comparison of body weight increase in rats injected pre- and postnatally with NGF antibodies (■), 6-hydroxydopamine (●), rabbit IgG (▲) or 0.15 M NaCl (x). Each curve represents the average weight of 20 rats per group.

using the procedure of Stoeckel *et al.*⁸—affinity chromatography of the IgG fraction on Sepharose 4B with covalently linked 2.5 NGF. After absorption and washes as described, NGF antibodies were eluted with 4.5 M $MgCl_2$, dialysed extensively against twice distilled water and lyophilized. These purified preparations of NGF antibodies contained no detectable IgG fraction cross-reacting with the renin-like activity present in mouse salivary gland extract⁹ and gave one single precipitin band in double-diffusion agar plates.

Female Sprague-Dawley rats were mated and at 16–17 days of gestation divided into four experimental groups. Pregnant rats were anaesthetized with ether, a middle incision made on the abdomen and the left and right uterine tubes exposed in sterile conditions. Each fetus of the first group (I) was injected subcutaneously with 40 μ g of purified NGF antibodies dissolved in 20 μ l of 0.15 M NaCl, using a 30-gauge needle inserted through the intact tube. The other groups served as controls and were injected with 20 μ l of 0.15 M NaCl (group II), 40 μ g of rabbit IgG (group III) or (group IV) with the dopamine derivative, 6-hydroxydopamine (6-OHDA), which in neonatal rodents produces massive destruction of para and pre-vertebral sympathetic ganglia^{10,11}. Of the 29 pregnant rats, one of group I and one of group II died. In group I, two rats underwent abortion and a third had to have its litter delivered through caesarian intervention 2 days after the end of the gestation time. All neonatal rats of the four groups appeared vital and compared favourably at birth with untreated litters. The only

noticeable difference to the untreated gestant rats was that the delivery of the litters was delayed by 24 h and in two cases by 48 h, with no apparent consequence for the mothers and the neonatal rats. The studies reported here are based on the analysis of 126 pups of the first group, 124 of the second, 48 of the third and 45 of the fourth group. In all neonatal rats the treatment was resumed immediately after birth at progressively higher doses according to the increase in body weight, as described previously¹².

Towards the end of the first postnatal week, pups injected with purified NGF antibodies (group I) were markedly smaller than those of the other three groups. 18 neonatal rats of group I died at the end of the first week and 22 before the end of the second week. In all instances death was preceded by progressive decrease in vitality and refusal to suckle even if they were taken care of by the mothers. To ensure that the weakest pups had access to nipples, the largest litters were reduced to nine pups by making available foster mothers deprived of their offspring. Six pups injected with saline (group II) and two treated with rabbit IgG (group III) died before the end of the second week. Three infants injected with 6-OHDA (group IV) died during the second postnatal week. The pups of groups II, III and IV developed normally although the latter were vital but slightly smaller than those of groups II and III (Fig. 1c). The 86 surviving rats treated with NGF antibodies were markedly smaller than all age-matched rats from the other three groups, as shown in Fig. 1a–c. Moreover, they exhibited sluggish, apathetic behaviour and moved only a few steps when exposed to strong stimuli, immediately resuming a static position, and did not show the exploratory behaviour characteristic of normal 2–4-week-old pups. On external inspection, their leg muscles appeared severely reduced in size. The behaviour of group I pups during resting of motility phases was strikingly similar to that of hypothyroid rats. Furthermore, they differed from those of other groups in their very weak reaction to pressure, pricking, burning or other noxious stimuli. Their body temperature to the hand was markedly lower than that of controls.

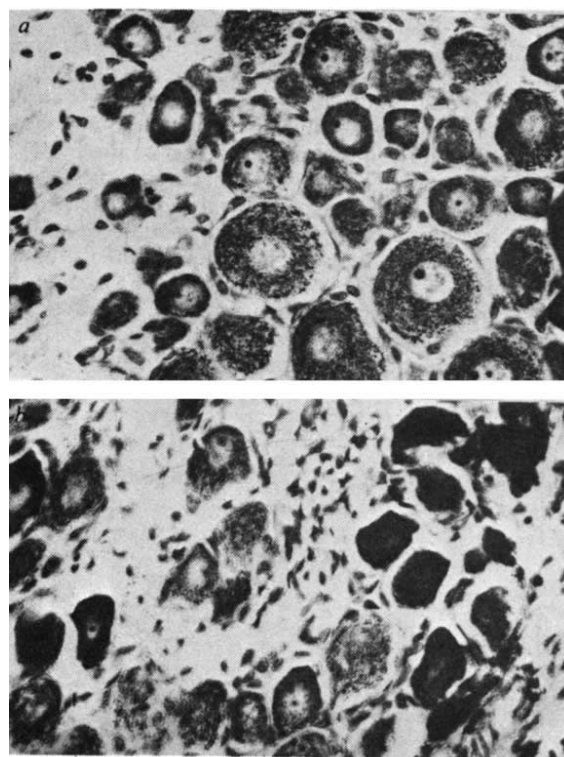


Fig. 2 Photomicrographs of sections through spinal sensory ganglia at the lumbar level of 9-day-old rats pre- and postnatally treated with 0.15 M NaCl (a) or NGF antibodies (b). Notice hypotrophic sensory nerve cells in the NGF antibody-treated rats. $\times 350$.

Histological studies of serially sectioned sympathetic, sensory ganglia, adrenal and extra-adrenal chromaffin cells gave the following results. At birth, sympathetic ganglia were already reduced to sclerotic nodules consisting of glial cells, fibroblasts and few small atrophic sympathetic neurones. The effect was similar but more pronounced and much more precocious than that elicited by AS-NGF in neonatal rats and mice⁴. The massive degeneration of chromaffin adrenal and extra-adrenal cells has been discussed previously¹³. Sensory ganglia, which do not exhibit morphological or functional alterations in postnatal AS-NGF-injected rodents¹⁴, appeared markedly reduced in size in 2–3-week-old group I pups. Figure 2 compares histological sections of sensory neurones of spinal ganglia of groups I and II. Nerve cells of the first group show various degrees of atrophy, ranging from decrease in cell size to evident signs of degeneration and piknosis. These findings provide evidence of severe damage to sensory neurones in rats treated with purified NGF antibodies during pre- and postnatal periods.

We also studied the accumulation of purified NGF antibodies in different organs and tissues. Fetuses, neonatal and 12-day-old rats were injected with unlabelled NGF antibodies and trace amounts of ¹²⁵I-NGF antibodies (1×10^6 c.p.m. per fetus or infant rat, specific activity 30,000 c.p.m. ng⁻¹). Fetuses and pups were killed 5 and 24 h later. Brain segments, hypophysis, kidneys, adrenals, salivary glands, liver, placenta, sensory and sympathetic ganglia were dissected out and the TCA-precipitable radioactivity counted. In fetuses the distribution of labelled antibodies was comparable in all organs tested, except for sympathetic and sensory ganglia, adrenal glands and hypophysis where the radioactivity was markedly higher. In a typical experiment, the specific radioactivity (counts per mg wet weight) 24 h after injections was 722, 222, 240 and 219 in sympathetic ganglia, adrenal glands, sensory ganglia and hypophysis, respectively, while the average value in all other organs, including brain regions, was 60 ± 20 . In animals injected after birth, the overall level of ¹²⁵I-NGF antibody distribution did not differ significantly from one organ to another, except in all brain segments where the radioactivity level was about one order of magnitude lower than in animals injected before birth, probably as a result of the development of the brain-blood barrier.

Our findings suggest that neutralization of NGF or a NGF-like molecule by NGF antibodies during a critical prenatal period produces damages to sensory nerve cells, as well as to sympathetic or chromaffin cell precursors¹³, and results in a syndrome reminiscent of human dysautonomic neuropathies. These findings support the hypothesis that this syndrome may result from a genetically produced NGF deficiency^{15–20}. Decrease in body weight and size, and sluggish and apathetic behaviour of infant rats injected during fetal life with NGF antibodies could be due to a direct or indirect damage to one or more components of the neuroendocrine system. A NGF role in the central nervous system has been previously hypothesized^{21–24}. Similar results were reported^{25,26} while this paper was in preparation.

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- Levi-Montalcini, R. & Boeder, B. *Proc. natn. Acad. Sci. U.S.A.* **42**, 384–391 (1960).
- Cohen, S. *Proc. natn. Acad. Sci. U.S.A.* **46**, 302–311 (1960).
- Levi-Montalcini, R. & Angeletti, P. U. *Pharmac. Rev.* **18**, 619–628 (1966).
- Steiner, G. & Schönbaum, E. *Immunosympathectomy* (Elsevier, Amsterdam, 1972).
- Levi-Montalcini, R. *Nerve Cells, Transmitters and Behaviour*, 84 (Elsevier, Amsterdam, 1980).
- Levi-Montalcini, R., Aloe, L., Calissano, P. & Cozzari, C. *Abstr. 3rd Meet. int. Soc. dev. Neurosci.*, Strasbourg, 5–6 (1980).
- Bocchini, V. & Angeletti, P. U. *Proc. natn. Acad. Sci. U.S.A.* **64**, 787–794 (1969).
- Stoeckel, K., Gagnon, C., Guroff, G. & Thoenen, H. *J. Neurochem.* **26**, 1207–1221 (1976).
- Cozzari, C., Angeletti, P. U., Lazar, J., Orth, H. & Cross, F. *Biochem. Pharmac.* **22**, 1321–1327 (1973).
- Angeletti, P. U. & Levi-Montalcini, R. *Proc. natn. Acad. Sci. U.S.A.* **65**, 114–121 (1970).
- Angeletti, P. U. & Levi-Montalcini, R. *Archs ital. Biol.* **108**, 213–221 (1970).
- Levi-Montalcini, R., Aloe, L., Mugnaini, E., Oesch, F. & Thoenen, H. *Proc. natn. Acad. Sci. U.S.A.* **72**, 595–599 (1975).
- Aloe, L. & Levi-Montalcini, R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1246–1250 (1979).
- Levi-Montalcini, R. *Harvey Lecture* **60**, 217–259 (1966).
- Riley, C. M. & Moore, R. H. *Pediatrics* **37**, 435–446 (1966).
- Pearson, J., Pytel, B. A., Grover-Johnson, N., Axerod, F. & Dancis, J. *J. neurol. Sci.* **35**, 77–92 (1978).
- Dolkart Gorin, P. & Johnson, E. M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5382–5386 (1979).
- Schwartz, J. P. & Breakefield, K. O. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1154–1158 (1980).

- Otten, U., Goedert, H., Mayer, N. & Lembeck, F. *Nature* **287**, 158–159 (1980).
- Crain, S. M., Peterson, E. R., Leibam, M. & Schulman, H. *Expl Neurol.* **67**, 205–214 (1980).
- Freed, W. J. *Brain Res. Bull.* **1**, 393–412 (1976).
- Benowitz, L. I. & Shashona, V. E. *Brain Res.* **172**, 561–565 (1979).
- Walker, P., Weichsel, M. E., Fisher, D. A., Guo, S. M. & Fisher, D. A. *Science* **204**, 127–129 (1979).
- Otten, U., Baumann, J. B. & Girard, J. *Nature* **282**, 413–414 (1979).
- Gorin, P. D. & Johnson, E. M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5382–5386 (1980).
- Johnson, E. M. *et al. Science* **210**, 916–918 (1980).

Mosaic distribution of opiate receptors, parafascicular projections and acetylcholinesterase in rat striatum

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Although cell-stained sections have suggested heterogeneity in the neostriatum¹, more conclusive evidence has come from selective neurochemical staining for receptors, enzymes or neural connections. For example, opiate receptors in the rat striatum are concentrated in discrete islands and in a streak under the corpus callosum^{2,3}—regions that lack any visible morphological distinction. In the monkey, cortical afferent fibres terminate in intricate patterns⁴. In the cat striatum, variations in the intensity of acetylcholinesterase (AChE) staining⁵ coincide with complex mosaic patterns of striatal efferent neurones, with cortical afferent terminations⁶ and with enkephalin-like immunoreactivity⁷. In both cats⁸ and monkeys⁹, thalamic afferent fibres, from the parafascicular-centre median complex, terminate in discrete clusters. Even the intensely studied nigrostriatal dopamine pathway, previously assumed to project diffusely throughout the caudate, can be shown to be heterogeneous by pharmacological methods¹⁰. To extend this evidence, we decided to examine, on serial sections of the same rat striatum, four different histological markers of striatal heterogeneity. We now report that there is a precise mosaic 'fit' in striatal 'islands' of closely packed opiate receptors, vacancies in the termination of parafascicular projections and AChE-poor zones.

We have recently developed an *in vitro* autoradiographical method whereby cryostat-cut sections of unfixed rat brain can be attached securely to slides so that morphology is preserved, even after incubation in radiolabelled ligands to visualize drug and neurotransmitter receptors¹¹. These sections are also suitable for visualizing intra-axonal transport of ³H-labelled amino acids used in classical tract-tracing autoradiography¹² and for AChE enzyme histochemistry¹³. Accordingly a mixture of ³H-proline and ³H-leucine was injected through pipettes by iontophoresis into the thalamic parafascicular nucleus¹² of 13 Sprague-Dawley rats (weighing ~200 g). The animals were killed 10–12 days later, 11 by perfusion with 10% formol-saline and 2 by decapitation. Sections from 11 perfused rats were processed for autoradiographical visualization of labelled thalamostriatal projections as described elsewhere¹². The two unperfused rat brains were removed and frozen in isopentane at –30°C before sectioning at –14°C in the cryostat. Sections (25 µm thick) were pressed onto cold gelatin-coated slides and melted in place, dried at 0°C and stored at –15°C for several days. Alternate sections were incubated in a buffered solution at 4°C containing ³H-naloxone as described previously¹¹ or at 25°C for 30 min with 1 nM ³H-D-Ala-D-Leu-enkephalin in a solution containing 50 mM Tris (pH 7.4), 3 mM MnCl₂ and 2 µM GTP^{14,15}. The labelled sections were then fixed in hot paraformaldehyde vapours¹¹, dipped in Kodak NTB-2 emulsion, exposed at –15°C for 6–21 days and then developed for autoradiography¹². The remaining sections were fixed in vapours,

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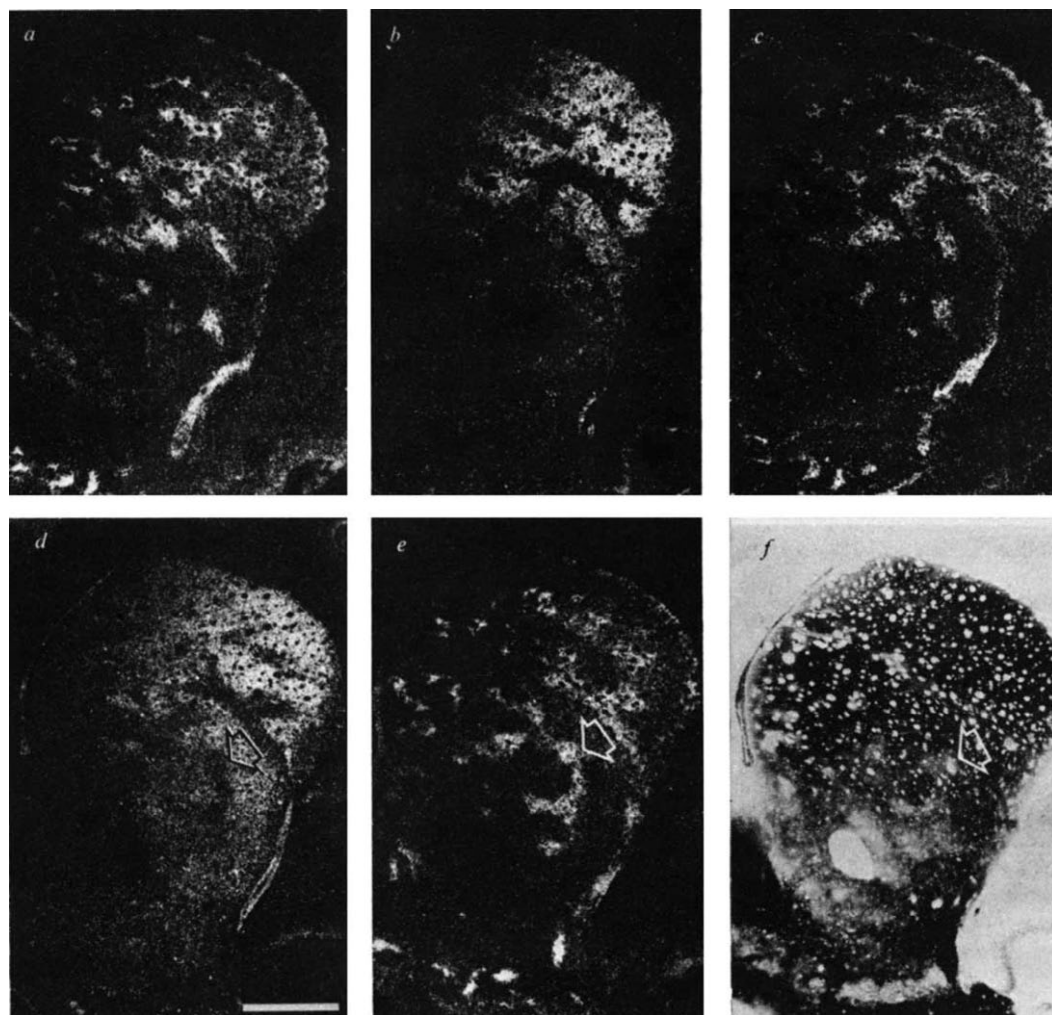


Fig. 1 Photographs of six sections taken from rostral levels of the caudate nucleus. Mid-line is to the left. *a, c, e* Are darkfield-illuminated autoradiographs of ^3H -naloxone binding visualized after the sections were exposed for 2 weeks to the emulsion. *b, d* Show the terminal distributions of thalamic parafascicular nucleus projections marked by ^3H -labelled amino acids in sections not treated for opiate binding but, instead, left exposed to the emulsion for 10 months. *f*, In brightfield illumination, had been stained for AChE according to a modification of the procedure described by Hardy *et al.*¹³—the sections were unfixed before AChE staining, and Iso-OMPA (K & K Laboratories, ICN) was used in place of promethazine in the incubation. Scale bar, 1 mm. Arrows point to corresponding sites exemplifying where receptor-dense zones are in register with both gaps in the thalamic termination and AChE-poor zones.

processed for autoradiography and allowed the lengthy exposure time, 6–10 months, required to visualize axonally transported label from the thalamus¹². Occasional sections were stained for AChE¹³ without prior fixation and subsequently briefly immersed in 10% formalin, stained with thionin, dehydrated and coverslipped.

Figure 1 shows that the parafascicular nucleus projections terminate throughout most of the striatum but conspicuously avoid certain irregularly shaped areas which correspond precisely to the dense opiate receptor clusters visualized in the adjacent ^3H -naloxone-incubated sections. As in other species¹⁶, AChE staining in rat striatum is uneven and contains AChE-

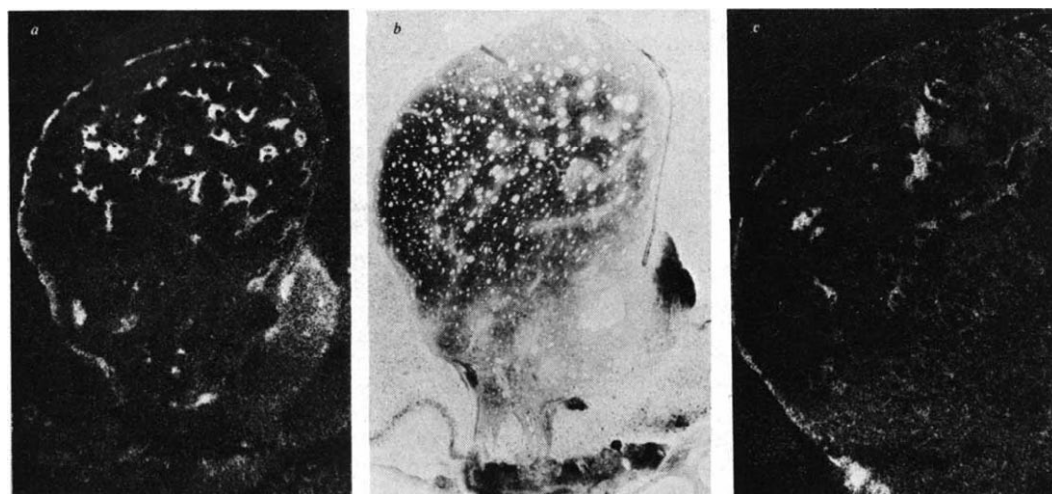
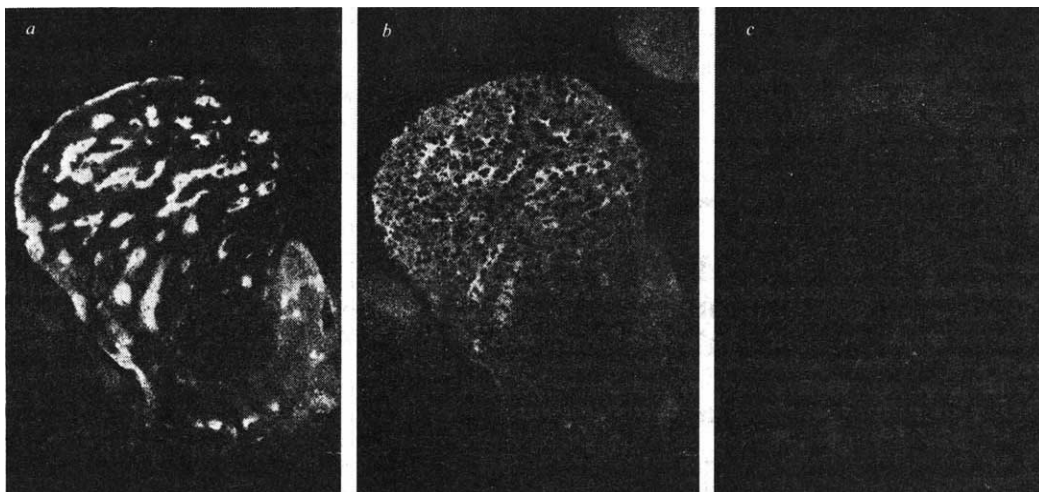


Fig. 2 *a*, Opiate receptor distribution at mid-levels of caudate. The visible streak lies immediately subjacent to the corpus callosum, seen as the dark band above. The diffuse and rather dense patch medially lies in the accumbens nucleus. A section about 200 μm removed from *a* is shown in *b*, stained for AChE. Note the similar degree of complexity and the sub-callosal streak for AChE-poor zones. In adjacent sections these would appear in register. In contrast to that pattern of correspondence, AChE-dense zones lie in the dorsomedial sector of the accumbens and in a tiny but clearly visible segment in the ventrolateral corner of the caudate nucleus—restricted zones which are also rich in opiate receptors. *c* Shows ^3H -naloxone binding to opiate receptors in caudal levels of striatum, where the globus pallidus and basal forebrain areas form the surrounding territories. In all three photographs medial is to the right. Magnification as in Fig. 1.

Fig. 3 *a, b*, Two adjacent sections incubated in ^3H -naloxone and ^3H -D-Ala-D-Leu-enkephalin, respectively. The opiate receptors labelled by the peptide are diffusely distributed throughout the striatum. When 10^{-6} M etorphine is included in either incubation medium, autoradiographical grains (white areas) are not visible. The section in *c* was incubated with 10^{-6} M etorphine and ^3H -D-Ala-D-Leu-enkephalin.



poor zones that coincide with striatal areas containing opiate receptor patches, although the diffuse edges and difficulty in controlling the intensity of the reaction make this concordance less dramatic. Similarly, the dense stripe of ^3H -naloxone-labelled opiate receptors situated just beneath the corpus callosum marks a zone that is correspondingly poor in both AChE activity and parafascicular projections. At rostral levels of striatum, ^3H -naloxone-labelled opiate receptors, parafascicular projections, and the AChE distribution mark corresponding zones that are rather large and diagonally orientated (Fig. 1). At mid levels of striatum, the opiate receptor distribution shows very striking borders and forms somewhat smaller patches than were visualized more rostrally (Fig. 2*a*), and the distribution of AChE is accordingly more intricate (Fig. 2*b*). In caudal striatum, the sub-callosal streak of ^3H -naloxone-labelled opiate receptors is still present, though narrower, and the receptor patches are smaller, often elongated and more widely separated (Fig. 2*c*). At these levels, gaps in the parafascicular projection and in AChE staining are hardly noticeable. However, wherever faint gaps can be observed they do correspond with the dense patches or stripes of receptors in adjacent sections. The receptor patches at all levels do not correlate with visible aspects of morphology, such as cell clusters¹.

In striatal sections incubated with ^3H -D-Ala-D-Leu-enkephalin, the labelling pattern is diffuse throughout striatal neuropil (Fig. 3) and, like ^3H -naloxone-labelled receptors, is absent in surrounding septum and corpus callosum. In addition, more densely labelled zones are coincident with ^3H -naloxone labelling observed in adjacent sections. A similar diffuse distribution of ^{125}I -D-Ala-D-Leu-enkephalin in rat striatum was recently described¹⁷ and termed the δ receptor distribution pattern after the terminology of Lord *et al.*¹⁸. However, we have recently shown that ^3H -D-Ala-D-Leu-enkephalin can bind in the patchy as well as the diffuse pattern depending on the incubation conditions used¹⁵, suggesting that ' μ ' and ' δ ' are functionally different conformations of the same, type I (ref. 14), opiate receptor.

In rat striatum, muscarinic receptor distribution¹⁹ shows a marked heterogeneity in the neonatal animal which becomes progressively more homogeneous in the first postnatal weeks. In contrast, ^3H -naloxone-labelled opiate receptors are densely and diffusely distributed in the prenatal rat and form clusters in the first postnatal week, whereas ^3H -D-Ala-D-Leu-enkephalin-labelled receptors appear later in their homogeneous adult pattern²⁰. The apparent loss of striking patterns of muscarinic receptor distribution as development proceeds may be due to the use of labelling conditions which are non-selective for muscarinic receptor subtypes²¹. With both opiate and muscarinic receptors, one receptor subtype with a clustered pattern may develop first, followed by a receptor subtype that 'fills in' to produce an apparently homogeneous adult pattern. Obviously, careful analysis of adjacent sections of developing

brain for several features will be a valuable tool for analysing the sequence of development. To this end, we have found that in addition to the localization of neural connections, receptor subtypes and AChE as shown here, the peroxidase-anti-peroxidase procedure for visualizing neuropeptide-specific antibodies can be successfully performed if the sections are first briefly preincubated with 2% formalin in phosphate-buffered saline²².

The unfixed cryostat-cut sections can also be used concurrently for visualizing catecholamine histofluorescence²³. Several types of nigrostriatal motor behaviour in rats²⁴, as well as neurochemical events²⁵⁻²⁷, have been shown to be subject to concurrent dopaminergic and 'opiate' modulation, even though the precise neuroanatomical substrates for these interactions remain unclear. The sub-callosal and patchy pattern of a subset of striatal dopamine terminals which develop early, fluoresce brightly and resist depletion by pharmacological manipulations¹⁰, seems to coincide with that of μ -oid, type 1 opiate receptors. By contrast, the more homogeneous distribution of dopaminergic terminals in the adult corresponds to the more homogeneous distribution of opiate receptors visualized with ^3H -D-Ala-D-Leu-enkephalin (Fig. 3). As brightly fluorescent dopaminergic terminals in the peripheral nervous system are found at sites where dopamine and enkephalin are co-stored and co-secreted²⁸, it is possible that only the subset of striatal dopaminergic terminals which seems to coincide with ^3H -naloxone-labelled opiate receptors contains co-stored enkephalin—one of a number of plausible notions testable by the methods and strategy described here.

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- Mensah, P. L. *Brain Res.* **137**, 53-66 (1977).
- Pert, C. B., Kuhar, M. J. & Snyder, S. H. *Life Sci.* **16**, 1849-1854 (1975).
- Pert, C. B., Kuhar, M. J. & Snyder, S. H. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3729-3733 (1976).
- Goldman, P. S. & Nauta, W. J. H. *J. comp. Neurol.* **171**, 369-386 (1977).
- Graybiel, A. M., Ragsdale, C. W. & Moon Edley, S. *Expl Brain Res.* **34**, 189-195 (1979).
- Ragsdale, C. W. & Graybiel, A. M. *Soc. Neurosci. Abstr.* **5**, 78 (1979).
- Graybiel, A. M., Ragsdale, C. W., Yoneoka, E. S. & Elde, R. P. *Soc. Neurosci. Abstr.* **6**, 342 (1980).
- Royce, G. J. *Brain Res.* **146**, 145-150 (1978).
- Kalil, K. *Brain Res.* **140**, 333-339 (1978).
- Olson, L., Seiger, A. & Fuxe, K. *Brain Res.* **44**, 283-288 (1972).
- Herkenham, M. & Pert, C. B. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5532-5536 (1980).
- Herkenham, M. *J. comp. Neurol.* **183**, 487-518 (1979).
- Hardy, H., Heimer, L., Switzer, R. & Watkins, D. *Neurosci. Lett.* **3**, 1-5 (1976).
- Pert, C. B., Taylor, D. P., Pert, A., Herkenham, M. A. & Kent, J. L. in *Neural Peptides and Neuronal Communication* (eds Costa, E. & Trabucchi, M.) 581-589 (Raven, New York, 1980).
- Bowen, W. D., Gentleman, S., Herkenham, M. & Pert, C. B. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Graybiel, A. M. & Ragsdale, C. W. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5723-5726 (1978).
- Goodman, R. R., Snyder, S. H., Kuhar, M. J. & Young, W. S. III *Proc. natn. Acad. Sci. U.S.A.* **77**, 6239-6234 (1980).
- Lord, J. A. H., Waterfield, A. A., Hughes, J. & Kosterlitz, H. W. *Nature* **267**, 495-499 (1977).
- Potter, A., Field, P. M. & Raisman, G. *Brain Res. Rev.* **1**, 185-205 (1979).
- Kent, J. L., Pert, C. B. & Herkenham, M. *Devl Brain Res.* (in the press).
- Birdsall, N. J. M., Hulme, E. C. & Burgen, A. *Proc. R. Soc. A* **207**, 1-12 (1980).

22. Keefer, D. A. *Cell Tissue Res.* **209**, 167-175 (1980).
23. de la Torre, J. C. & Surgeon, J. W. *Histochemistry* **49**, 81-93 (1976).
24. Pert, A. in *Characteristics and Function of Opioids* (Developments in Neuroscience Vol. 4) (eds Van Ree, J. M. & Terenius, L.) 389-401 (Elsevier, Amsterdam, 1978).
25. Iwatsubo, K. & Clouet, D. H. *Biochem. Pharmacol.* **24**, 1499-1503 (1975).
26. Hong, J. S., Yang, H.-Y. T., Fratta, W. & Costa, E. *J. Pharmacol. exp. Ther.* **205**, 141-147 (1978).
27. Parenti, M., Gentleman, S. & Neff, N. H. *Fedn Proc.* **39**, 516 (1980).
28. Hökfelt, T. *et al.* in *Neural Peptides and Neuronal Communication* (eds Costa, E. & Trabucchi, M.) 1-23 (Raven, New York, 1980).

Cell-surface antigen distinguishes sensory and autonomic peripheral neurones from central neurones

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Cell-surface markers are useful in identifying and studying different cell types in the nervous system. For example, the major cell types in rat dorsal root ganglion (DRG) cultures have been identified using a combination of tetanus toxin and antibodies to the cell-surface antigens Ran-1 and Thy-1. Neurones bind tetanus toxin and express Thy-1, Schwann cells express only Ran-1 and fibroblasts express only Thy-1 (ref. 1). A natural extension of this approach was to use antibodies to distinguish between different neuronal subpopulations on the basis of their cell-surface antigens. We therefore immunized mice with cells from rat DRG cultures, and then used the hybridoma technique of Köhler and Milstein² to produce monoclonal antibodies. We report here an antibody that recognizes a surface antigen which is present on all rat peripheral neurones we have studied and absent from neurones derived from the central nervous system. An antigen with reciprocal distribution, expressed only by neurones of the rat central nervous system, has been defined by Cohen and Selvendran³.

DRG cultures from 5-day-old W/Fu or Sprague-Dawley rats were prepared as described previously¹. Cultures were enriched for neurones by treating with 10^{-5} M cytosine arabinoside after 1 day in culture for a period of 48 h. After 2 weeks, cells were washed with Dulbecco's minimal essential medium, scraped off three Falcon flasks containing 5×10^5 neurones each, and injected intraperitoneally (i.p.) into two BALB/c mice. One week later the mice were boosted i.p. using nine flasks and a total of 4.5×10^6 neurones.

When tested by indirect immunofluorescence (IIF) 3 days after the boost, serum antibodies to neurones, Schwann cells and fibroblasts in DRG cultures could be demonstrated (for method see Fig. 1 legend). Therefore, on this day, spleen cells from one mouse were fused with a non-immunoglobulin-secreting myeloma (NS-1) using polyethylene glycol⁴. Cells were plated out into two 24-well Linbro trays at 2×10^6 cells per well in HAT (hypoxanthine-aminopterin-thymidine) medium⁵. Hybridoma clones were screened for antibody production on 5-6-day-old rat DRG cultures grown for 2-14 days on collagen-coated coverslips by IIF on living cells. Hybrids producing antibodies that bound to the surface of DRG neurones were selected and cloned twice by limiting dilution on layers of peritoneal macrophages as described elsewhere⁶. One stable cell line, 38/D7, was selected for further study. The immunoglobulin class of the antibody was shown by immunofluorescence with class-specific antibodies to be IgM.

38/D7 antibodies specifically labelled neurones and not Schwann cells or fibroblasts in DRG cultures from 5-6-day-old W/Fu, Sprague-Dawley and hooded rats. Exceptionally, in older cultures, occasional flat cells stained. When cultures were prepared from adult rats, by a method described⁷ for adult mouse DRG neurones, the staining pattern was the same. Double immunofluorescence labelling showed that this anti-

body bound to the surface of all tetanus toxin-positive neurones in these cultures (Fig. 1a-c) although the intensity of staining on different cell bodies was variable. Developmentally, the antigen was first detected by double-label immunofluorescence with tetanus toxin in DRG cultures from 15-day-old rat embryos examined after 24 h *in vitro*. Cultures from 14-day-old embryos examined after 24 h *in vitro* showed no labelling.

Indirect immunofluorescence staining of primary cultures from the rat neonatal nervous system showed that peripheral neurones in the superior cervical ganglion, nodose ganglion and myenteric plexus (K. R. Jessen and R. Mirsky, unpublished observations) also bound 38/D7 antibodies. There was no staining in sciatic nerve cultures, which contain only Schwann cells and fibroblasts. Neurones in central nervous system (CNS) cultures from spinal cord (Fig. 1d-e), cerebellum, cerebrum and retina did not bind the antibody (Table 1). In the case of the cerebellum, cultures containing neurones were prepared from rats as old as 4 weeks. We have shown previously, using a wide variety of cell type-specific antibodies, that these cultures include Schwann cells, fibroblasts, astrocytes, oligodendrocytes, Muller cells, ependymal cells and leptomeningeal cells^{1,6,8}. In addition, myoblasts and myotubes in primary muscle cultures were negative, as were melanocytes in primary cultures from the skin of newborn hooded rats. Because 38/D7 antibodies bound only to neurones of the peripheral nervous system (PNS) we conclude that the antigen is absent from all other cell types in PNS and CNS cultures, including neurones from the CNS.

Interestingly, in dissociated cultures of rat adrenal medulla (prepared as described by Unsicker *et al.*)⁹ maintained in the presence of nerve growth factor (NGF), small clusters of noradrenaline-containing cells¹⁰, with the morphology of chromaffin cells, bound 38/D7 antibodies.

In addition, the rat pheochromocytoma cell line PC12, which is derived from an adrenal medulla chromaffin cell tumour¹¹, bound the antibody in the absence as well as the presence of NGF in conditions where it extends neurites. Neither the rat ethylnitrosourea-induced tumour lines B35, B50, B82 and B104, which are of CNS origin¹², nor the rat-mouse glioma-neuroblastoma hybrid NG108 (ref. 13), bound antibody.

We confirmed the antigen occurrence *in situ* using 38/D7 antibodies in conjunction with peroxidase-labelled rabbit anti-mouse Ig on fixed frozen sections of DRG and cerebellum. In adult rat DRG, fixed by perfusion with 4% paraformaldehyde, there was weak labelling of the edge of neuronal cell bodies. No labelling was seen in adult cerebellar sections (Fig. 2). It is often difficult to visualize surface antigens in frozen sections^{14,15}; here it was impossible to visualize 38/D7 binding in frozen sections using immunofluorescence, and difficult even with the peroxidase technique.

The relatively low levels of antibody binding seen with this monoclonal antibody in immunofluorescence and immunoperoxidase studies probably reflect low levels of antigen on the neuronal cell surface rather than a low binding constant for the

Table 1 The distribution of antigen recognized by 38/D7 antibodies in cultures of rat nervous tissue

Age of rat	Culture	Days <i>in vitro</i>	Labelled neurones
5-6 days	Dorsal root ganglion	2, 28	+
14-day embryo		1	-
15-day embryo		1	+
Adult		3	+
Newborn	Superior cervical ganglion	14	+
Newborn	Nodose ganglion	7	+
7-8 days	Myenteric plexus	8	+
5-6 days	Cerebellum	5	-
4 weeks	Cerebellum	3	-
10-day embryo	Cerebrum	3, 30	-
15-day embryo	Spinal cord	6, 16	-
Newborn	Retina	12	-

+, Antigen present; -, antigen absent.

antibody. Concentration of the antibody supernatant 10 times or use of Ascites fluid obtained by injecting 38/D7 hybridoma cells into a BALB/c mouse did not increase the intensity of staining on neurones. Furthermore, binding did not increase when DRG cultures were fixed with 2% paraformaldehyde to stabilize the antigen-antibody complex between incubations

with 38/D7 antibody and tetramethyl rhodamine-labelled goat anti-mouse Ig. The low levels of antibody binding made the use of quantitative assays and absorption studies to characterize the antigen tissue distribution difficult.

Immunofluorescence studies on PC12 cells stained in suspension showed that the binding of 38/D7 was substantially

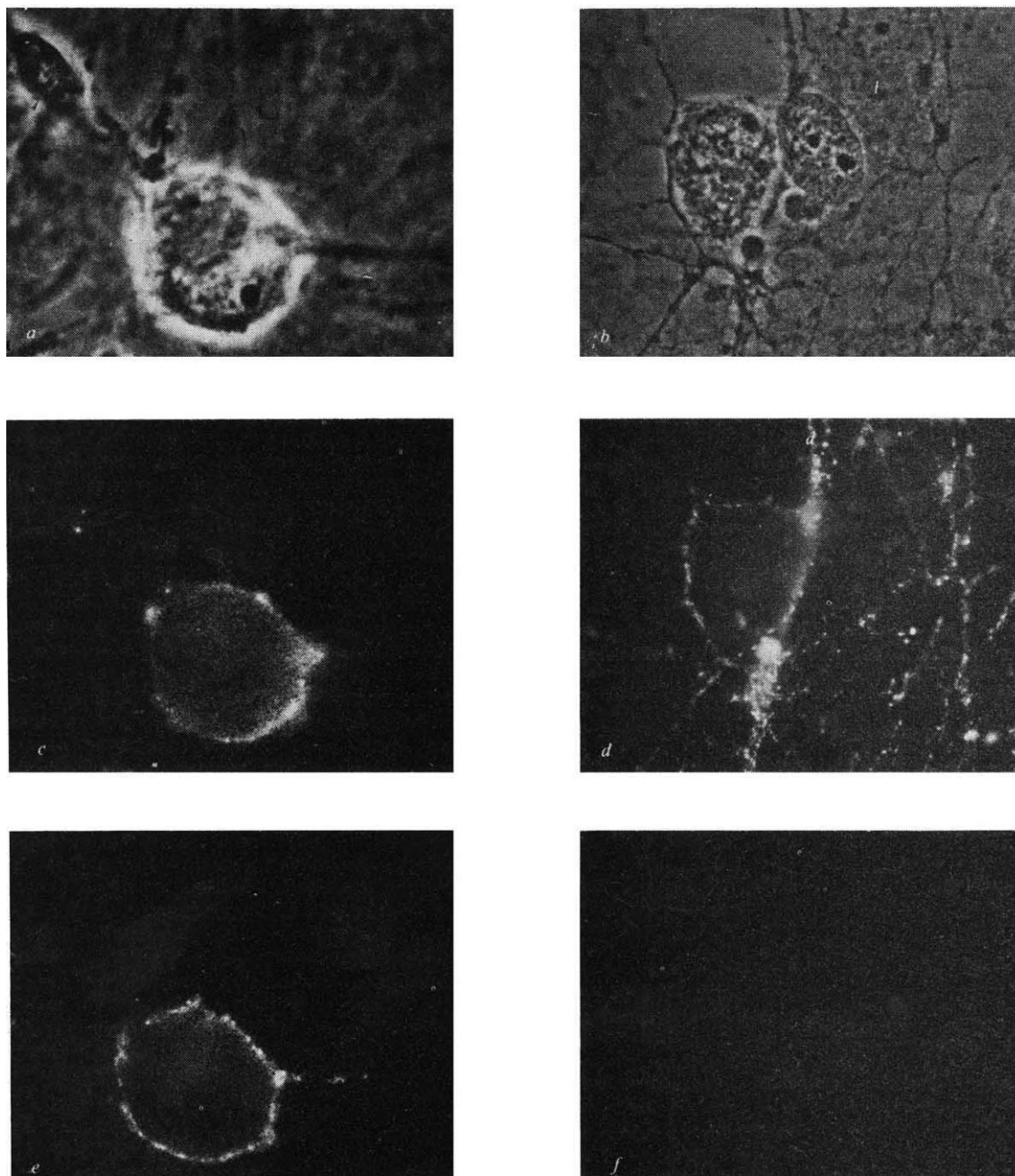


Fig. 1 38/D7 labelling of peripheral, but not central, neurones. *a*, Phase-contrast photomicrograph of a culture of rat DRG. *b*, Tetanus toxin, and *c*, 38/D7 antibodies label the neuronal cell body, as visualized by IIF; neurites are labelled, but are not in the plane of focus. Non-neuronal cells are not labelled in either case. *d*, Phase-contrast photomicrograph of a spinal cord culture. *e*, A neuronal cell body and neurites are labelled by tetanus toxin. *f*, There is no labelling by 38/D7 antibodies. Double immunofluorescent staining: cells on collagen-coated coverslips were incubated with $3\text{--}5\ \mu\text{g ml}^{-1}$ tetanus toxin diluted in minimal essential medium (MEM)-HEPES with 5% fetal calf serum (FCS) for 30 min at room temperature. After washing with MEM-HEPES, rabbit anti-tetanus and 38/D7 antibodies (neat hybridoma supernatant or ascites fluid (see text) diluted 1:100) were added in 40- μl volumes for 30 min at room temperature. Bound immunoglobulin was detected by further incubation for 30 min with fluorescein-conjugated goat anti-rabbit immunoglobulin (Nordic, diluted 1:50 in MEM-HEPES + 5% FCS) together with tetramethyl rhodamine-conjugated goat anti-mouse immunoglobulin (Cappel, diluted 1:100). The cells were then fixed in 5% acetic acid in ethanol for 20 min at -20°C , and mounted in glycerol with phenylenediamine and sealed with nail varnish. The slides were examined by phase-contrast and epifluorescent illumination with either fluorescein or rhodamine filters on a Zeiss microscope. Photographs were taken with high-speed Kodak Ektachrome colour slide film. Exposure times were 30–90 s for immunofluorescence and 1/4–1/15 s for phase-contrast images. $\times 2,300$.

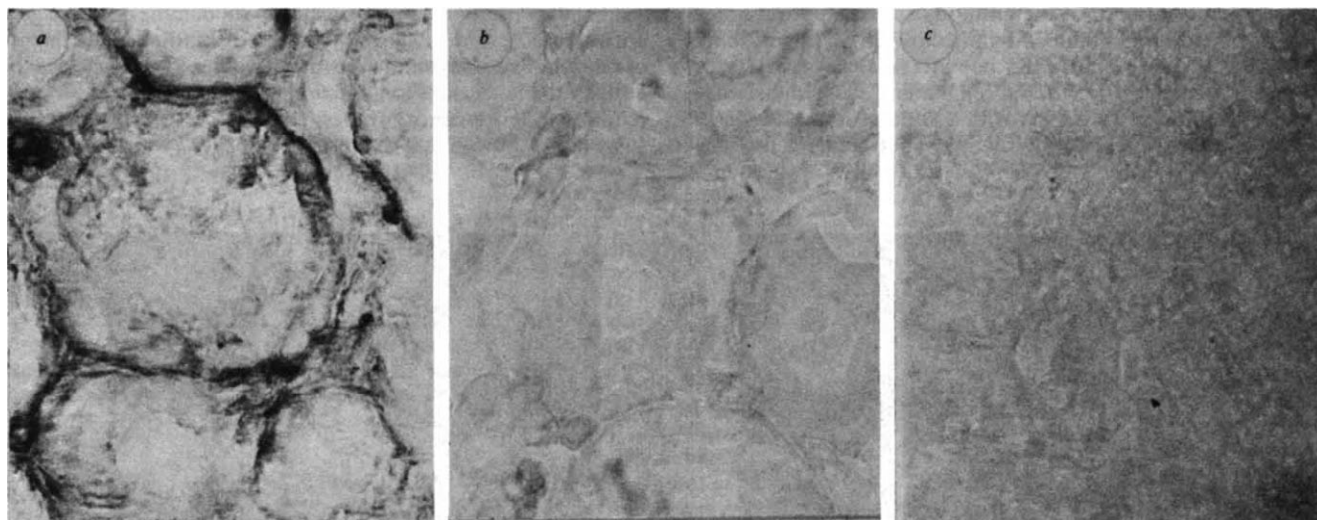


Fig. 2 38/D7 labelling of adult rat DRG neurones in section. *a*, Bright-field photomicrograph of a 10- μ m adult rat DRG frozen section incubated with 38/D7 antibodies. Note precipitate visible round the edges of the neurones, faint staining within the cell. *b*, Bright-field photomicrograph of 10- μ m adult rat DRG frozen section treated with A4 IgM hybridoma supernatant³ as control. Note the general absence of staining with no distinct precipitate visible around neuronal cell bodies. *c*, Bright-field photomicrograph of a 20- μ m adult rat cerebellum frozen section treated with 38/D7 antibodies. Note the general absence of staining round the Purkinje cell. Adult rat DRG were perfusion fixed with 4% paraformaldehyde, soaked overnight in 7% sucrose in phosphate-buffered saline (PBS), then frozen in liquid nitrogen. Sections (10 μ m) were cut at -20°C on a Brighton cryostat, air dried, then treated with 1% H_2O_2 in methanol for 15 min and washed in PBS. Sections were incubated with 38/D7 antibodies (neat hybridoma supernatant or ascites fluid (see text) diluted 1:100) or inactive hybridoma supernatant as a control for 30 min at room temperature, washed and then incubated with rabbit anti-mouse coupled to peroxidase (Nordic, diluted 1:100) for 30 min at room temperature. After washing in PBS and 0.05 M Tris-HCl pH 7.2, the sections were incubated in diaminobenzidine (0.5 mg ml⁻¹) and 0.03% H_2O_2 in 0.05 M Tris-HCl pH 7.2, washed, mounted in fluoromount and viewed by bright-field illumination with $\times 63$ objective in a Zeiss Universal microscope.

reduced after preincubation of the cells at 37°C for 30 min with trypsin, suggesting that the antigen is a protein.

Finally, in a preliminary investigation of the species distribution of the antigen, DRG cultures from mouse, chicken and human showed no antibody binding, suggesting that it is rat specific.

Thus, the monoclonal antibody we have raised binds to an antigen, which is probably a protein, on the cell surface of rat peripheral neurones but does not bind to CNS neurones. The antigen is expressed by sympathetic, NGF-dependent neurones of the superior cervical ganglion as well as sensory NGF-independent neurones of the nodose ganglion and DRG. Although the origin of nodose ganglion neurones in the rat is unknown, in the chick they are of placodal origin¹⁶, which indicates that the antigen may not be restricted to neurones derived from the neural crest. Crest-derived cells in the adrenal medulla and the pheochromocytoma PC 12 also express the antigen, whereas satellite cells and Schwann cells in various ganglia and melanocytes, which are also crest derived, do not. As the antigen is not expressed by neurones in DRG cultures until these reach an age equivalent to 16–17 days *in utero*, it can be considered a developmental antigen.

In this and the accompanying paper³, antibodies which distinguish antigens present on the surface of neurones in the CNS and PNS are described for the first time. Tetanus toxin specifically binds to the surface of both central and peripheral neurones in culture¹⁷, while Thy-1 is expressed on the surface of DRG neurones and on 10% or less of tetanus toxin-positive neurones in cultures of cerebellum, corpus callosum and cerebral cortex^{1,8}. Nervous system-specific antigens defined by conventional antisera, which are present on the surface of neurones or neuroblastomas, have been described by several authors^{18–22}. More recently, monoclonal antibodies specific to human fetal brain and various human nervous system tumours²³, to chick neurones in both the CNS and PNS²⁴, and to some rat retinal neurones, CNS glial cells and rat brain²⁵ have been described.

The two monoclonal antibodies raised in this laboratory against neurones identify different cell-surface antigens; these

allow us to distinguish all CNS neurones from all PNS neurones. There may be several surface molecules that are restricted in expression either to neurones derived from the neural tube, or to neurones derived from the neural crest and placode. We find it difficult to imagine what the function of such molecules could be.

We thank the people in this neuroimmunology group, particularly Erika Abney, Jim Cohen, Jelena Gavrilovic, Mark Noble, Martin Raff and Janet Winter for help and advice, and Durward Lawson and Roy Mahoney for advice and help with photography.

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- Fields, K. L., Brookes, J. P., Mirsky, R. & Wendon, L. M. B. *Cell* **14**, 43–51 (1978).
- Köhler, G. & Milstein, C. *Eur. J. Immun.* **6**, 511–519 (1976).
- Cohen, J. & Selvendran, S. Y. *Nature* **291**, 421–423 (1981).
- Galfre, G., Howe, S. C., Milstein, C., Butcher, G. W. & Howard, J. C. *Nature* **266**, 550–552 (1977).
- Littlefield, J. W. *Science* **145**, 709 (1964).
- Bartlett, P. F. *et al. Brain Res.* (in the press).
- Scott, B. J. *Neurobiol.* **8**, 417–427 (1977).
- Raff, M. C. *et al. Brain Res.* **174**, 283–308 (1979).
- Unsicker, K., Krisch, B., Otten, U. & Thoenen, H. *Proc. natn. Acad. Sci. U.S.A.* **75**, 3498–3502 (1978).
- Furness, J. B. & Costa, M. *Histochemistry* **41**, 335–352 (1975).
- Greene, L. A. & Tischler, A. S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2424–2428 (1976).
- Schubert, D., Heinemann, S., Carlisle, W., Tarikas, H. & Kimes, B. *Nature* **249**, 224–227 (1974).
- Nelson, P., Clifford, C. & Nirenberg, M. *Proc. natn. Acad. Sci. U.S.A.* **73**, 123–127 (1976).
- Moore, M. J., Dikkes, P., Reif, A. E., Romanul, F. C. A. & Sidman, R. L. *Brain Res.* **28**, 283–293 (1971).
- Gervais, A. G. *Folia-biol., Praha* **18**, 50–52 (1972).
- Akeson, R. & Narayanan, Y. *Anat. Rec.* **196**, 71–82 (1980).
- Mirsky, R., Wendon, L. M. B., Black, P., Stolkin, C. & Bray, C. *Brain Res.* **148**, 251–259 (1978).
- Rutishauser, U., Thierry, J.-P., Brackenbury, R. & Edelman, G. M. *J. Cell Biol.* **79**, 371–381 (1978).
- Rutishauser, U., Gail, W. E. & Edelman, G. M. *J. Cell Biol.* **79**, 382–393 (1978).
- Jorgensen, O. S. & Bock, E. *J. Neurochem.* **23**, 879–880 (1974).
- Akeson, R. & Seeger, R. C. *J. Immun.* **118**, 1995–2003 (1977).
- Schachner, M., Ruberg, M. Z. & Carnow, T. B. *Brain Res.* **11**, 367–377 (1976).
- Kennett, R. H. & Gilbert, F. *Science* **203**, 1120–1121 (1979).
- Eisenbarth, G. S., Walsh, F. S. & Nirenberg, M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4913–4917 (1979).
- Barnstable, C. J. *Nature* **286**, 231–235 (1980).

A neuronal cell-surface antigen is found in the CNS but not in peripheral neurones

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In embryogenesis, the neural tube gives rise to the neurones of the central nervous system (CNS) whereas those in the peripheral nervous system (PNS) are generated from the neural crest and possibly also the placodes. Little is known of the part played by cell-surface molecules in this early sorting-out process and in subsequent interactions between different classes of neurone during development. Of the few known antisera that seem to define neuronal cell-surface antigens, none appears to distinguish between neuronal subpopulations¹⁻⁴. However, the discriminating power of the immunological approach to this question has been greatly enhanced by the advent of the hybridoma technique for making monoclonal antibodies⁵⁻⁸. We have produced a hybridoma secreting an antibody that binds to the surfaces of neurones from the CNS but not from the PNS. The accompanying letter describes a monoclonal antibody with the reciprocal neuronal distribution⁹.

Rat cerebellar cells prepared by trypsin digestion of tissue from 1-week-old Wistar-Furth rats as previously described¹⁰ were grown in dissociated cell culture for 4-7 days in minimal Eagle's medium (MEM) containing 10% fetal calf serum (FCS, Gibco), 2.5% chick embryo extract (Flow) and 24 mM KCl, on poly-L-lysine (PLL, Sigma)-coated glass coverslips in 24-well trays (Linbro) or in PLL-coated plastic flasks (Falcon) and maintained in 95% air-5% CO₂. The cultures were enriched for neurones by growing them after 1 day in culture in the continuous presence of cytosine arabinoside (10⁻⁵ M, Sigma), a mitotic poison which selectively kills dividing non-neuronal cells. Previous marker studies¹¹ have established that cerebellar cultures grown in these conditions consist primarily of three cell types—neurones, astrocytes and fibroblasts. Cells for immunization were scraped off the culture flask in 15 mM HEPES-buffered MEM (MEMH), washed once in MEMH and 10 × 10⁶ cells were injected intraperitoneally (without adjuvant) into female BALB/c mice. One week later the mice received a booster injection of 10 × 10⁶ cells. When tested by indirect immuno-

Table 1 Fluorescence labelling of cells in various primary cultures with monoclonal antibody A4

Rat tissue	Neurones	Non-neuronal cells
Cerebellum	+	—
Cerebrum	+	—
Spinal cord	+	—
Hippocampus	+	—
Olfactory bulb	+	—
Retina	+	—
Dorsal root ganglion	—	—
Superior cervical ganglion	—	—
Nodose ganglion	—	—
Sciatic nerve	—	—
Skeletal muscle	—	—

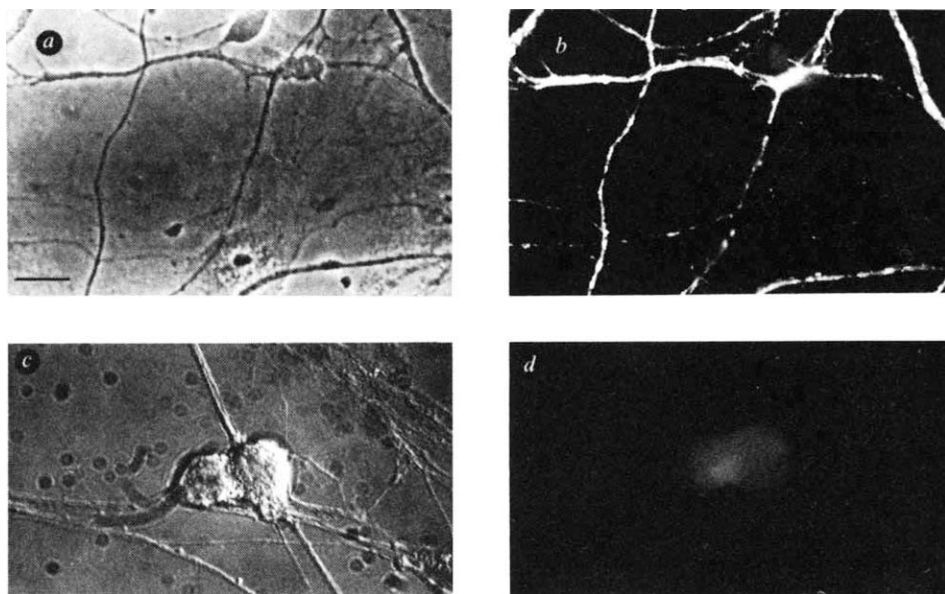
Rat tissue cultures were prepared as described in the text or ref. 11 from 0-6-day-old rat tissues and grown *in vitro* for 4-6 days. Except in cases where neurones could be unambiguously identified on morphological grounds, they were recognized by double immunofluorescence assays using tetanus toxin followed by both antibody A4 and F(ab')₂ rabbit anti-tetanus toxin. The binding of monoclonal antibody was visualized with rhodamine-conjugated goat anti-mouse immunoglobulin and the rabbit antibody with fluoresceinated goat anti-rat immunoglobulin. Previous marker studies¹¹ have established that in these cultures the non-neuronal cells include astrocytes, oligodendrocytes, fibroblasts, ependymal cells, Müller cells, leptomeningeal cells, Schwann cells and microglia.

fluorescence (IIF), sera taken 24 h later were found to label all cell types in coverslip cultures of cerebellum. At the same time these were titred in an indirect radioimmunoassay (RIA) against cerebellar cells grown in the PLL-coated wells of a 96-well microtitre tray (Linbro).

6 × 10⁷ spleen cells from the mouse with the highest serum titre were fused 3 days after the booster immunization with 15 × 10⁶ P3-NS1/1-Ag4-1 (NSI) cells in the presence of polyethylene glycol as described by Galfre *et al.*¹². After fusion the cells were distributed in 7 wells of a 24-well tray (Linbro) in RPMI containing 15% FCS, sodium pyruvate (100 mM) and hypoxanthine, aminopterin and thymidine (HAT medium), fed with fresh HAT medium twice weekly for 2 weeks followed by two changes in HT medium (HAT minus aminopterin), and thereafter in RPMI/FCS.

Supernatant solutions from cultures showing vigorous hybrid cell growth (five out of seven wells) were assayed 2 weeks after the fusion by IIF and RIA against cultured cerebellar cells. Two of the five uncloned hybridoma supernatants showed relatively high binding activity (up to 10 times background) in RIAs. Only one of these, A4, will be described here.

Fig. 1 A4 binding to rat CNS but not PNS neurones. Cells in cultures of 6-day-old rat cerebella (a, b) or dorsal root ganglia (c, d) were labelled after 5 days in culture with antibody A4 (1:100 dilution of immune ascites from a mouse carrying a peritoneal A4-hybridoma tumour), followed by rhodamine-conjugated goat anti-mouse immunoglobulin (G anti-MIg-Rd, Cappel; 1:100), fixed in acetic acid-ethanol (5:95) and then viewed with phase contrast (a) or Nomarski (c) and fluorescence (b, d) optics. Note that cerebellar neurones and their processes, but not the underlying fibroblastic cells, are labelled in b whereas the prominent sensory neurones in d are A4 negative. Scale bar, 10 µm.



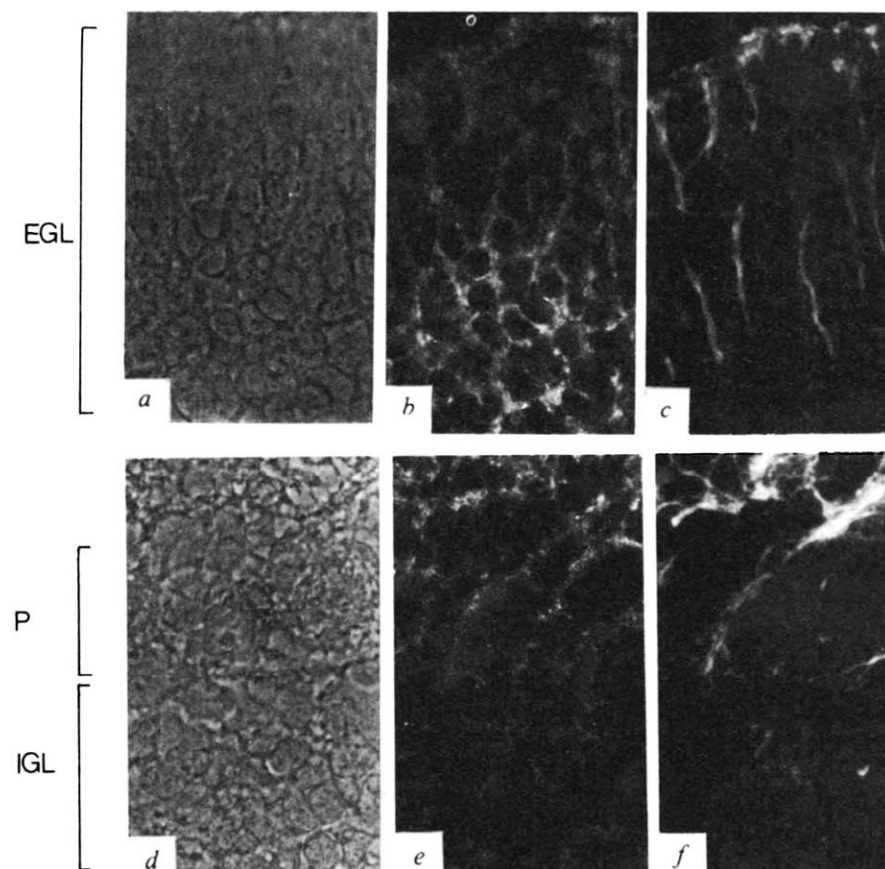


Fig. 2 Localization of A4 binding in adolescent (16-day-old) cerebellum. Small blocks of freshly dissected cerebellum were frozen in isopentane at -196°C . Sections ($6\text{ }\mu\text{m}$) were cut on a cryostat at -15°C and then incubated simultaneously with antibody A4 (supernatant 1:4) and rabbit anti-glial fibrillary acidic protein antiserum (R α GFAP, 1:200). After washing, binding was visualized by incubating with G anti-RIg-Rd (1:100) and sheep anti-mouse immunoglobulin fluorescein (1:100). Sections were viewed by phase (a, b) or fluorescence (b, c and e, f) optics. b, e, Antibody A4; c, f, R α GFAP; EGL, external granular layer; P, Purkinje layer; IGL, internal granular layer.

The A4 hybridoma was cloned by limiting dilution on feeder layers of mouse peritoneal cells. A4 clones continued to secrete antibody and after recloning twice, these were grown either in culture for medium collection or passaged intraperitoneally in irradiated BALB/c mice. Immune ascites from A4 tumour-bearing mice showed binding activity when diluted up to 10^7 times and was at least 100 times more potent than culture fluid. Antibody A4 was shown by Ouchterlony immunoprecipitation with class-specific reagents to be an IgM antibody.

Immunofluorescence assays of cerebellar cultures showed that antibody A4 staining was confined to the surfaces of cells with neuronal morphology (Fig. 1a, b). This neuronal staining was confirmed in double-labelling experiments with tetanus toxin, a neuronal-specific cell-surface marker¹. A4 staining and toxin binding overlapped completely: all tetanus toxin⁺ cells were labelled with A4 and vice versa (data not shown). The distribution of A4 binding on individual neurones was also comparable with that seen with toxin; the surfaces of cell perikarya and neurites along their entire length were stained (Fig. 1b).

The cell type distribution of the antigen recognized by antibody A4 was further examined by immunofluorescence labelling of cultures from a wide range of neonatal rat central and peripheral nervous tissue (Table 1). In dissociated cell cultures of spinal cord, retina, hippocampus, cerebrum and olfactory bulb, prepared as described for cerebellum^{10,11}, double-labelling studies showed that in all cases A4 staining and tetanus toxin binding coincided completely in cell type specificity. Thus, antibody A4 seems to recognize an antigen shared by all neurones in the CNS. By contrast, the antigen was absent from all cell types including neurones in cultures of rat dorsal root (Fig. 1c, d) sympathetic cervical and nodose ganglia. No cells stained in cultures of rat sciatic nerve, which contain Schwann cells and fibroblasts but no neurones¹³ or skeletal muscle. Of the various cell lines tested, including the rat-mouse glioma-neuroblastoma hybrid NG108 (ref. 14), the ethylnitrosourea-induced rat tumour line B104 (ref. 15), and the rat pheochromocytoma cell line PC12 (ref. 16), none bound A4.

The distribution of antigen *in vivo* was examined by IIF in $6\text{-}\mu\text{m}$ cryostat sections of immature cerebellum (postnatal day 16). Sections were double-labelled with both antibody A4 (Fig. 2b, e) and rabbit anti-glial fibrillary acidic protein (Fig. 2c, f), an intracellular glial specific marker¹¹, to contrast the pattern of radial glial staining with the neuronal specificity of antibody A4

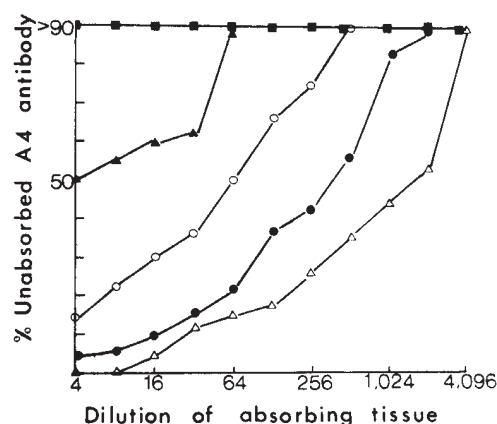


Fig. 3 Quantitative absorption analysis of A4 immune ascites with rat CNS tissue of various ages. A4 immune ascites ($50\text{ }\mu\text{l}$, 1:250) was added to $50\text{-}\mu\text{l}$ portions of doubling dilutions of 50% (v/v) tissue homogenates in MEMH and incubated for 45 min on ice. After centrifugation, the residual antibody A4 in aliquots ($50\text{ }\mu\text{l}$) of supernatant was assayed for binding to microwell cultures of 6-day-old rat cerebella (text) using ^{125}I -labelled rabbit anti-mouse μ heavy chain ($50\text{--}100,000\text{ c.p.m. per well}$) as second antibody. The washed microtitre plates were cut up and individual wells assayed for bound radioactivity in a γ -counter. Ordinate, residual antibody A4 expressed as a percentage of the maximal binding obtained with unabsorbed immune ascites (1:500). Each point is the mean of duplicate determinations in a single representative experiment. ■, 8-day-old whole embryo; ▲, 10-day-old embryo brain; ○, new born cerebellum; ●, 7-day-old cerebellum; △, adult cerebellum.

binding. With A4, ring fluorescence was clearly seen around most of the cell bodies in the external granular layer (Fig. 2b), and with lower intensity around the large Purkinje perikarya and small cells in the internal granular layer (Fig. 2e). No intracellular staining was seen with A4 and white matter (not shown) was only weakly positive. Control sections of rat dorsal root ganglion were unstained by this antibody (not shown, but see Fig. 2b in ref. 9).

The time of appearance and accretion of the antigen during rat CNS development was determined in quantitative absorption assays with homogenates of brain or cerebellum. Immune ascites fluid (1:500) was absorbed with doubling dilutions of homogenates from rats of various ages and residual antibody estimated in RIAs on cultured cerebellar cells (Fig. 3). The antigen was absent from 8-day-old fetuses but was detectable in 10-day-old embryo brain although at <1% of the adult level. By birth the concentration in brain had increased 30-fold (data not shown). Cerebellum, which develops postnatally in rodents, had 3% of adult levels at birth and 25% by day 7. The adult concentration was reached at 3 weeks (not shown).

Using the same quantitative absorption assay, several non-neural rat tissues were tested for the presence of antigen. Levels of <1% of the adult brain concentration were recorded in thymus, bone marrow, red blood cells, testes, liver and muscle (data not shown). Spleen had 3% of the adult brain level, but here binding of A4 was nonspecific. Thus, in IIF experiments with cryostat sections of adult rat spleen and brain staining with the monoclonal antibody was blocked by preincubation with normal goat serum in the case of spleen but not brain. Thus, we conclude that the antigen defined by the A4 monoclonal antibody is CNS specific.

The extent of species cross-reactivity of the antigen was examined by absorbing immune ascites with CNS tissue homogenates from chicken, frog, mouse, guinea pig, rabbit, sheep, pig, cow and human. The antigen was absent from chicken and frog and among the mammals from mouse (strains tested included BALB/c, CBA, AkR, C57 and B6) and guinea pig. However, human, rabbit, ovine, bovine and pig CNS tissue had levels of antigen comparable with those found in rat.

The exact chemical nature of the antigen remains unknown. Preliminary experiments indicate that the molecule is resistant to digestion (1 h at 37°C) by trypsin (10 mg ml⁻¹), pronase (10 mg ml⁻¹) and neuraminidase (50 µg ml⁻¹). However, the molecule is completely destroyed by heating to 100°C for 5 min. Deoxycholate treatment of rat brain membranes successfully solubilizes the antigen and experiments are in progress with the aim of purifying the antigen on antibody affinity columns to facilitate more detailed chemical studies.

Therefore, monoclonal antibody A4 defines a cell-surface molecule common to all CNS neurones but absent from all neurones in the PNS. Measurable amounts of the antigen are found in rat CNS as early as the 10th embryonic day and antigen rapidly accumulates in parallel with the processes of neurogenesis and neuronal maturation. In the immature cerebellum, the molecule is present on neuronal precursors including germinal cells. Thus, A4 represents a valuable new marker for studies of mammalian CNS neuronal differentiation both in culture and *in vivo*, with several advantages over tetanus toxin, the most useful cell-surface marker for neurones so far described¹. Moreover, preliminary experiments have shown (E. Abney, B. Brown and M. Raff, unpublished observations) that antibody A4 is cytotoxic for CNS neurones in the presence of complement. This should allow exploration of the effects of selectively depleting A4⁺ neurones on the subsequent differentiation and interactions in culture of non-neuronal cells (such as astrocytes and oligodendrocytes). The antibody should also facilitate the purification by affinity methods of A4⁺ neurones with the aim of establishing homogeneous cultures of neurones from the CNS. The functional role of the antigen is at present obscure. An assessment of the ability of the antibody to perturb normal neuronal functions and/or differentiation, both *in vivo* and in culture, should help to elucidate this problem.

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Note added in proof: Recent studies using cultures of optic nerve from 6-day-old rats have identified a subpopulation of A4⁺/GFAP⁺ process-bearing cells. Interestingly, the morphology of these cells closely resembles that of the tetanus toxin⁺/GFAP⁺ optic nerve astrocytes described by Raff *et al.*¹¹.

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1. Mirsky, R., Wendon, L. M. B., Black, P., Stolkin, C. & Bray, D. *Brain Res.* **148**, 251–259 (1978).
2. Rutishauser, U., Thierny, J.-P., Brackenbush, R. & Edelman, G. M. *J. Cell Biol.* **79**, 371–381 (1978).
3. Rutishauser, U., Gall, W. E. & Edelman, G. M. *J. Cell Biol.* **79**, 382–393 (1978).
4. Jorgensen, O. S. & Møller, M. *Brain Res.* **194**, 419–429 (1980).
5. Köhler, G. & Milstein, C. *Nature* **256**, 495–497 (1975).
6. Kennet, R. H. & Gilbert, F. *Science* **203**, 1120–1121 (1979).
7. Eisenbarth, G. S., Walsh, F. & Nirenberg, M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4913–4917 (1979).
8. Barnstable, C. J. *Nature* **286**, 231–235 (1980).
9. Vulliamy, T., Ratnay, S. & Mirsky, R. *Brain Res.* **291**, 418–420 (1981).
10. Cohen, J., Dutton, G. R., Wilkin, G. P., Wilson, J. E. & Balazs, R. J. *Neurochemistry* **23**, 899–900 (1974).
11. Raff, M. C. *et al. Brain Res.* **174**, 283–308 (1979).
12. Galfré, G., Howe, S. C., Milstein, C., Butcher, G. W. & Howard, J. C. *Nature* **266**, 550–552 (1977).
13. Brookes, J. P., Fields, K. L. & Raff, M. C. *Nature* **266**, 364–366 (1977).
14. Nelson, P., Clifford, C. & Nirenberg, M. *Proc. natn. Acad. Sci. U.S.A.* **73**, 123–127 (1976).
15. Schubert, D. *et al. Nature* **249**, 224–227 (1974).
16. Greene, L. A. & Tischler, A. S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2424–2428 (1976).

Non-random openings and concentration-dependent lifetimes of glutamate-gated channels in muscle membrane

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Our preliminary results¹ with a single concentration (10⁻⁴ M) of L-glutamate suggested that a glutamate receptor-gated channel (glutamate channel) in locust extrajunctional muscle membrane could suddenly change its kinetic properties and switch from a short to a long lifetime mode. These results were obtained from denervated muscle² pretreated with the lectin concanavalin A^{3,4} (Con A) to block glutamate receptor desensitization. We have since extended our patch clamp studies of locust muscle to cover a range of glutamate concentrations and to include innervated muscle and muscle which has not been treated with Con A. These new studies show that the lifetime of the glutamate channel depends on the concentration of glutamate in the patch electrode, which we explain by a multi-binding site receptor model. This model, and our finding that channel openings occur non-randomly, also accounts for the apparent transitions in channel lifetime described in our earlier publication.

Single glutamate channels associated with D-receptors were recorded from the extrajunctional membrane of innervated metathoracic extensor tibiae muscle fibres in adult locusts (*Locusta migratoria*) using the extracellular patch clamp technique^{1,5–10}. Fresh pipettes were used for each concentration of glutamate (10⁻⁶–10⁻² M). Recordings from Con A-treated muscle fibres were made after pretreatment with 1–2 µM Con A for ~15 min. Experiments were performed at the muscle resting potential (–55 to –60 mV) and at ~20°C. The open-close kinetics of single channels from muscle preparations which had not been pretreated with Con A were determined over a glutamate concentration range of 7.5 × 10⁻⁵–7.5 × 10⁻⁴ M.

Figure 1 is a frequency histogram of the number of opening events, seen at an extrajunctional site and presumably involving a single receptor ionophore complex, in successive 5-s time intervals over a recording period of 300 s. Variation in this

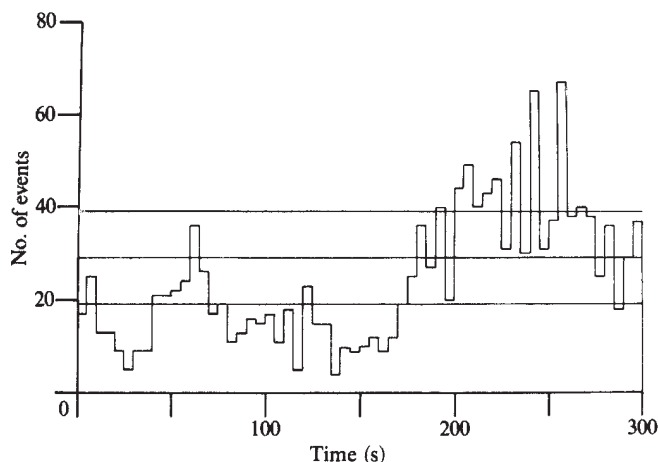


Fig. 1 Frequency histogram of the number of opening events in successive 5-ms time intervals determined from a single glutamate channel with 10^{-5} M glutamate in the patch electrode. A sample of the record from which these data were obtained is presented in Fig. 2a. The middle horizontal line represents the mean number of events per time bin, the upper and lower lines indicating 95% confidence limits for a Poisson distribution. Resting potential, -60 mV; temperature, $\sim 20^\circ\text{C}$. See text for further explanation.

frequency was a consistent feature of our recordings from Con A-treated muscles. Clearly the observed number of openings in most time bins lies outside the Poisson predicted limits. With concentrations of L-glutamate between 10^{-6} M and 10^{-4} M the intervals between openings of single glutamate channels recor-

ded from Con A-treated preparations are usually exponentially distributed, suggesting that openings of single channels might be randomly distributed in time. However, exponential interval distributions are necessary, but not sufficient, to show that a process is Poisson and they are not very sensitive to deviations from randomness^{11,12}. To determine more definitively whether the frequency of channel openings conforms to a Poisson process, the number of events occurring in equal, consecutive and non-overlapping time intervals were counted. The probability (P) of a given number of events (x) being observed in a particular interval of time (Δt) is given by:

$$P\Delta t = \frac{e^{-m} m^x}{x!}$$

where m = mean number of events in interval Δt . In a Poisson distribution, the mean of the distribution equals its variance (v). Mean values of the ratio v/m with, for example, 10^{-5} , 5×10^{-5} and 10^{-4} M glutamate were 2.3 ± 1.52 (s.d.), 2.38 ± 1.23 and 3.72 ± 3.6 respectively. We tested the significance of deviations of v from m for different Δt s using a simple t -test:

$$t(N-1 \text{ degrees of freedom}) = \frac{(v/m) - 1}{\text{s.e.}}$$

where N = number of samples and standard error (s.e.) = $(2/N-1)^{1/2}$. With concentrations of L-glutamate between 10^{-6} M and 10^{-4} M, values of v/m were all significantly ($P < 0.001$) greater than the value of unity expected for a Poisson distribution. (Data from over 100 recording sites on a sample of 40 preparations studied over 15 months.) Similar results were obtained with higher concentrations of glutamate but their

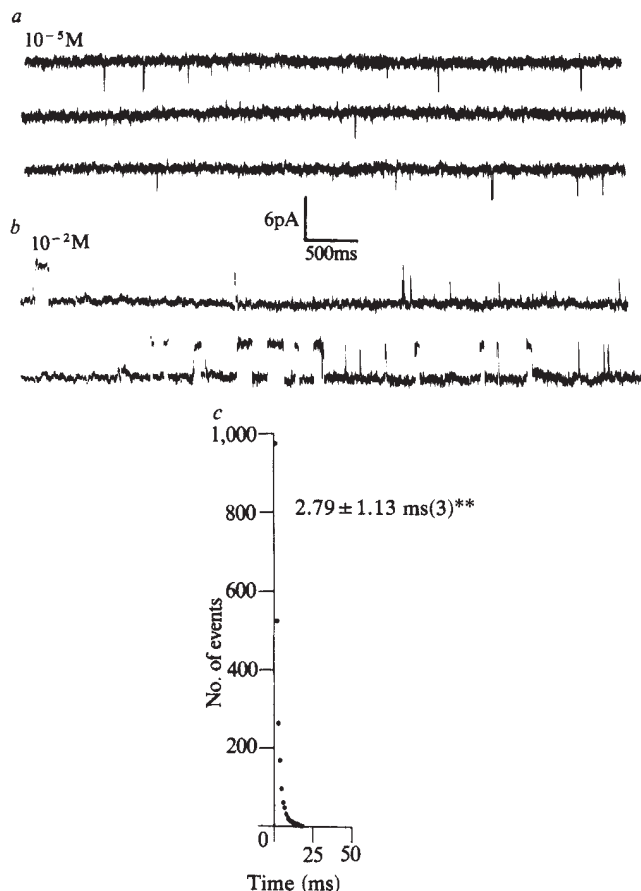


Fig. 2 Effect of glutamate concentration on channel lifetime. *a, b*, Single channels recorded in the presence of two different concentrations of L-glutamate, as indicated. Channel openings are downward excursions of traces for both recordings. Note channel seen with 10^{-2} M glutamate is mainly in the open state. Both records were obtained from muscle fibres which had been pretreated with Con A (10^{-6} M) to block desensitization. Resting potential, -60 mV; temperature, $\sim 20^\circ\text{C}$. *c-e*, Frequency histograms of channel lifetimes recorded in the presence of *c*, 5×10^{-5} M, *d*, 10^{-3} M, and *e*, 5×10^{-3} M glutamate. Lifetimes were measured from digitized data as the difference between the first downward deflection of the single channel current and the next upward deflection. The histograms show a progressive increase in the proportions of long open events with increasing glutamate concentration. ** Mean channel lifetime $\pm$ s.d.

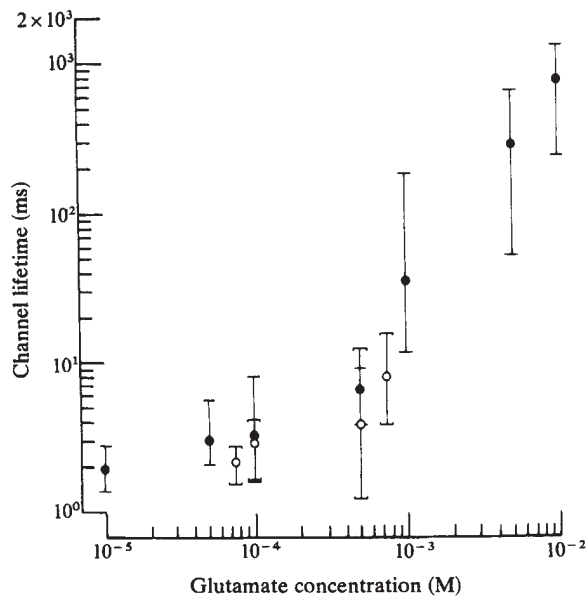


Fig. 3 Log-log plots of mean channel lifetime against glutamate concentration, obtained from Con A-pretreated (non-desensitizing) muscle preparations (●) and non-treated (desensitizing) preparations (○). Each datum point represents the average of the mean channel lifetimes of at least three recording sites (the filled circle datum point at 10^{-4} M was derived from recordings at over 50 sites). The vertical bars indicate the ranges of mean channel lifetimes for each glutamate concentration studied.

interpretation is complicated by the increased mean channel lifetime at these high concentrations. Values of v/m did not increase with increasing concentrations of glutamate between 10^{-6} M and 10^{-4} M. This suggests that non-random opening of single channels is unlikely to have resulted from unsuspected residual desensitization following treatment of the preparations with Con A, although we cannot completely exclude this possibility.

It is difficult to explain the non-random activity of single glutamate channels without invoking temporal variations either in the affinity of ligand binding site(s) or in the steps leading to ionophore gating or in the environment of the receptor-ionophore complex. Support for non-random opening of single receptor-gated channels comes from recent studies of single channel activity in planar membranes seeded with acetylcholine receptors isolated from *Torpedo* electroplax¹³, where, in the presence of carbamylcholine, a slight but significant deviation from random channel opening in time was noted¹⁴.

We have previously inferred^{1,5,6} that with 10^{-4} M L-glutamate, single channel activity of 6-day denervated muscle is characterized by two or more populations of channel lifetimes, but the present studies covering a range of glutamate concentrations (10^{-6} – 10^{-2} M) and using innervated rather than denervated muscles make this unlikely. At 10^{-5} M glutamate, channel lifetimes were rarely >12 ms, whereas at the other end of the concentration range studied lifetimes >1 s were observed (Fig. 2). Histograms of frequency distributions of channel lifetimes at various glutamate concentrations are illustrated in Fig. 2c–e, showing that the range of channel lifetimes increased with concentration of glutamate and that this was accompanied by a reduction in the proportion of channel lifetimes <10 ms. Although these trends were apparent over the entire range of concentrations studied, their impact on mean channel lifetime was not significant until the concentration exceeded $\sim 10^{-4}$ M (Fig. 3). Because of the impossibility of fitting exponentials to many of the histograms we have calculated mean channel lifetimes directly from measured data. The frequency of single channel openings also increased with glutamate concentration although this parameter was obviously depressed at the higher

concentrations due to the appearance of long channel lifetimes.

We have now excluded the possibility that long channel lifetimes may result from pretreatment of the muscle fibre with Con A by demonstrating their occurrence at extrajunctional sites on muscles which have not been exposed to this lectin. With glutamate concentrations ranging from 7.5×10^{-5} M to 7.5×10^{-4} M, the frequency of channel openings recorded from preparations not treated with Con A was significantly depressed compared with preparations exposed to this lectin¹⁵ and channel openings often occurred in bursts interposed between long closed periods. Nevertheless, with the higher concentrations ($>10^{-4}$ M) of glutamate in the patch electrode this activity was characterized by long open events of the type observed at sites on Con A-treated muscles.

There remains the possibility that the long channel lifetimes result from high-frequency serial openings of normal duration separated by brief closed periods that could not be resolved due to the limited frequency response of our recording system. Our patch clamp data are usually recorded through a low-pass filter with a 3 Db cut-off frequency of 1 kHz (ref. 1) when we can reliably detect channel fluctuations of duration >300 μ s at sites with good signal-to-noise ratios ($\sim 3:1$). At the best recording sites one can resolve channel activity without introducing a low-pass filter into the recording circuit. In these conditions there is a 3-Db cut-off frequency of ~ 3 kHz and we can detect $\sim 50\%$ of channel fluctuations (openings and closings) as brief as 50 μ s and 100% of fluctuations ≥ 100 μ s duration. Our recording system was calibrated using 5-pA square pulses (50 μ s–2 ms). Even in the absence of a low-pass filter, records of channel activity obtained with high concentrations of L-glutamate in the patch electrode were still characterized by long open events.

The balance of evidence supports the contention that channel lifetime depends on the L-glutamate concentration. A receptor incorporating two or more binding sites for L-glutamate each capable of gating the receptor ionophore and with the constraint that all sites must be vacated for ionophore closure to occur would satisfactorily explain this relationship. The increased frequency of glutamate binding which would be expected to accompany an increased concentration of glutamate, would raise the probability of overlapping occupancy of the postulated binding sites on the glutamate receptor. This would result in an increase in the mean channel lifetime. The non-randomness of channel openings suggests that the affinity of the binding site might change with time. With low concentrations of agonist this non-randomness is manifested as fluctuations in the frequency of channel openings; with higher concentrations of glutamate our model would predict transient increases in the simultaneous occupancy of the binding sites and the appearance of long channel lifetimes. The model also predicts a continuous relationship between mean channel lifetime and glutamate concentration, a prediction borne out by our studies (Fig. 3).

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1. Patlak, J. B., Gration, K. A. F. & Usherwood, P. N. R. *Nature* **278**, 643–645 (1979).
2. Usherwood, P. N. R. *Nature* **223**, 411–413 (1969).
3. Mathers, D. A. & Usherwood, P. N. R. *Nature* **259**, 409–411 (1976).
4. Mathers, D. A. & Usherwood, P. N. R. *Comp. Biochem. Physiol.* **59C**, 151–155 (1978).
5. Neher, E., Sakmann, B. & Steinbach, J. H. *Pflügers Arch. ges. Physiol.* **375**, 219–228 (1978).
6. Usherwood, P. N. R., Clark, R. B., Gration, K. A. F., Ozeki, M. & Patlak, J. J. *Physiol., Paris* **75**, 615–621 (1979).
7. Clark, R. B., Gration, K. A. F. & Usherwood, P. N. R. *J. Physiol., Lond.* **301**, 60P–61P (1979).
8. Gration, K. A. F. *Neurotox. 1979 Symp. Soc. Chem. Ind.*, 169–179 (1980).
9. Gration, K. A. F., Lambert, J. J. & Usherwood, P. N. R. *Proc. 14th int. Physiol. Soc. Budapest* (in the press).
10. Usherwood, P. N. R. *INSERM Symp.* **13**, 367–383 (1980).
11. Hubbard, J. I., Llinas, R. & Quastel, D. M. J. *Electrophysiological Analysis of Synaptic Transmission* (Arnold, London, 1969).
12. Usherwood, P. N. R. *J. Physiol., Lond.* **227**, 527–551 (1972).
13. Nelson, N., Anholt, R., Linstrom, J. & Montal, M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3057–3061 (1980).
14. Schindler, H. & Quast, U. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3052–3056 (1980).
15. Gration, K. A. F., Lambert, J. J. & Usherwood, P. N. R. *J. Physiol., Lond.* **310**, 49P (1980).

Sodium channels need not open before they inactivate

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Most, if not all, types of voltage-gated channels inactivate. Channels that have been driven open by a depolarization will enter a non-conducting, inactivated state if the depolarization is maintained long enough. This inactivated state is different from the resting closed state because inactivated channels cannot be forced open by depolarization. Opinion about the mechanism of inactivation has ranged from Hodgkin and Huxley's original supposition that the inactivation process proceeds independently of events leading to channel opening¹⁻³ to the more recent view that a channel normally must open before inactivating^{4,5}. Intermediate theories suppose that activation processes—but not necessarily the actual opening of channels—are more or less tightly coupled to inactivation⁶⁻⁹. We now provide evidence, based on recordings of currents flowing through single sodium channels, that channel opening is not a prerequisite for inactivation to occur, and that inactivation seems to proceed independently of activation.

We have used the method of recording single sodium channel currents, introduced by Sigworth and Neher¹⁰ and modified by R.H. and J.P.¹¹, so that transmembrane potential is accurately known. Briefly, a fire-polished glass microelectrode with a tip pore opening about 0.5 μm in diameter is pressed on to the surface of a rat myotube grown in tissue culture conditions. When good electrical contact between the muscle surface membrane and the glass electrode is achieved, negative pressure is applied to the electrode so that the muscle membrane is drawn partly into the aperture of the electrode and forms a seal to the glass. After a good seal has developed, the electrode is quickly withdrawn from the muscle surface, and the bath solution is simultaneously changed from normal Ringer's to a solution (160 mM CsF, 5 mM Cs HEPES, pH 7.3) appropriate for the cytoplasmic surface of the membrane. The patch of membrane in the electrode aperture adheres to the glass, so the final arrangement is a small area of membrane separating two electrolyte-filled compartments (inside and outside the electrode). Resistance between these compartments is about $10^{10} \Omega$. Voltage across the isolated membrane can be controlled, and current flowing can be measured with a resolution determined by the background electrical noise of about 0.3 pA r.m.s. at a 1-kHz bandwidth. Conclusions presented here are based on observations of six different membrane patches. Individual patches usually yield useful data for between 20 and 60 min and sometimes last for >90 min. The mean open time of channels, in the conditions of the experiments described here, is about 3 ms, and the shortest openings that can be detected are about 0.15 ms. We estimate that perhaps 5% of the openings are too short to be detected. Our patches generally contained three to five channels.

We have tested the predictions made by two distinct hypotheses: first, that inactivation can only occur after a channel has opened, and second, that inactivation proceeds independently of channel opening. These two hypotheses make quite different predictions about the behaviour of channels that by chance have not opened promptly after the membrane patch was depolarized. Specifically, we have estimated the probability $P(t)$ that a channel is open t seconds after the beginning of the depolarizing stimulus, and also a conditional probability $w(T, t)$ that a channel is open at time t , given that no opening was observed for an interval of T seconds after the start of a depolarization. If channels must open before inactivation can occur, the conditional probability function should look like the

overall probability of opening function, except that it should be shifted in time by T seconds; that is, $w(T, t+T) = P(t)$. The argument is that inactivation should, under our first hypothesis, proceed along a time course determined by when the channel first opens, no matter how long one happens to wait for that opening.

If inactivation occurs independently of channel opening, however, the conditional probability function should rise to a value smaller than the peak of $P(t)$ —some inactivation has occurred during the T seconds' wait when the channel happened not to open—and the falling phase of the conditional probability function should coincide with that of the overall opening probability function; that is, $w(T, t) = P(t)$ for time long enough after T that channels have had a chance to open. When inactivation is not coupled to opening, the amount of inactivation present after T should be the same whether or not the channel happened to open before T . Briefly, we have found that the conditional probabilities behave as if inactivation occurred independently of channel openings.

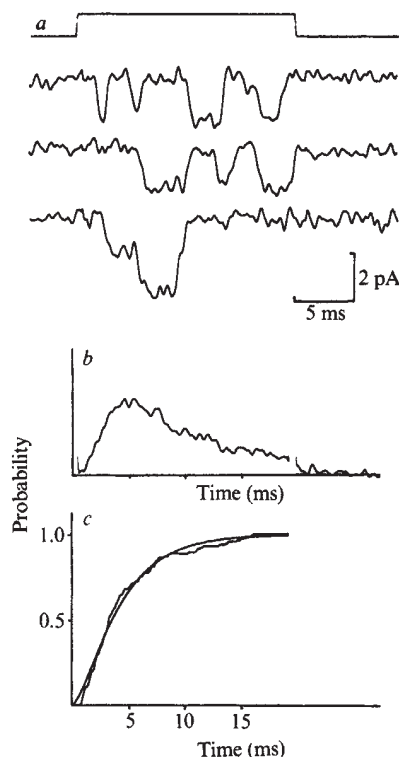


Fig. 1 Single-channel recordings in an excised patch. *a* Shows three individual records from a set of 144 depolarizations to -40 mV from a holding potential of -90 mV. The voltage pulse is depicted above the current traces. In the third trace the currents from two channels overlapped. *b* Shows an arbitrarily scaled and inverted sum of the currents from the same set of depolarizations. It estimates the probability density that a channel is open at a given time. In 27/144 traces of this series no channel openings were detected. *c* Plots the cumulative distribution of times after the voltage step to the first opening for the same set of voltage pulses. The smooth curve is a theoretical histogram for a sequential model of activation which has two closed states leading to an open state. The curve has the equation $P(t) = 1 + K_1 e^{-t/\tau_1} - K_2 e^{-t/\tau_2}$, where time constants $\tau_1, \tau_2 = 1.26, 3.46$ ms. The Ringer's solution in the pipette contained (in mM): 150 NaCl, 5 KCl, 1.5 CaCl_2 , 1 MgCl_2 , 5 glucose, 5 HEPES at pH 7.4. The cells were pretreated with $1 \mu\text{g ml}^{-1}$ α -bungarotoxin (Sigma) for 5 min before experiments to block acetylcholine receptors. Potassium channels were effectively blocked by Cs at the cytoplasmic surface. Leakage and capacity transients were subtracted from the traces by averaging the currents obtained by 25-mV hyperpolarizing voltage steps, then scaling and adding them to the currents induced by depolarization. Traces with no events were also averaged and subtracted from individual records to remove any residual capacity transients. All records were low-pass filtered at 1 kHz. Other experimental details were as described previously¹¹. Temperature, 8.9°C .

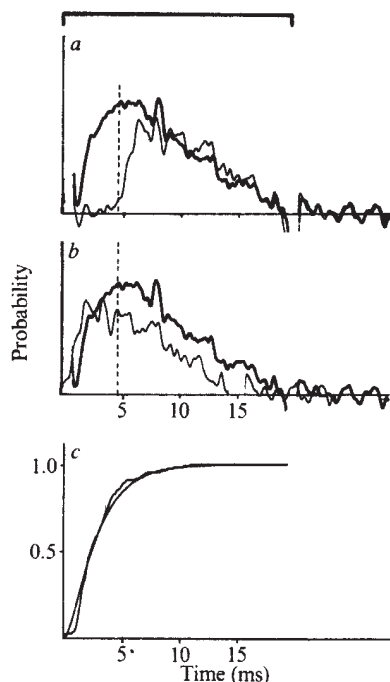


Fig. 2 Probabilities of channel opening. *a* Shows the probability (heavy line) $P(t)$ of a channel being open at a given time, as in Fig. 1*b*. Currents obtained by a 19.5-ms voltage step, indicated by the bracket at the top of the figure, to -40 mV from the holding potential of -119 mV. The records were averaged from 144 depolarizations. The patch contained three channels. The lighter line shows the average of all traces which had no openings during the first 4.55 ms, which is indicated by the dashed line. Note that the falling phase of this averaged current, which estimates the conditional probability $w(T, t)$ (see text), closely follows the falling phase of $P(t)$. *b* Replots the same data as *a*, and shows that the conditional probability, when shifted by 4.55 ms to the left, falls below the unconditional probability. *c* Plots the cumulative distribution of first latencies for this series of depolarizations. The theoretical curve has the time constants $\tau_1, \tau_2 = 0.91, 2.18$ ms; temperature 9.8°C .

Specimen records of single depolarization epochs shown in Fig. 1*a* confirm the observations described by Sigworth and Neher¹⁰: the single channel currents are about 1 pA in amplitude, and exhibit the behaviour expected of a channel with an open-shut gate that behaves stochastically. When a large number of records like that shown in Fig. 1*a* are averaged, the resulting function (Fig. 1*b*) gives an estimate of the probability $P(t)$ that a channel is open at each particular time, as long as channels are independent and identical and do not change their properties over the period of observation. Simple averaging estimates this probability because all open channels contribute the same current and thus serve as counters, each of which adds a unit to the total whenever the channel is open. The distribution of times to the first opening is found as shown in Fig. 1*c*.

Conditional probabilities $w(T, t)$ were calculated, and the result, superimposed on the overall probability $P(t)$, is illustrated in Fig. 2*a* for another patch. The distribution of times until first opening for this patch is shown in Fig. 2*c*, and the conditional probabilities $w(T, t)$ were calculated by averaging all records whose first open time exceeded 4.55 ms as indicated on the histogram. After the first 4.55 ms the conditional probability rises to a peak and then joins the overall probability function $P(t)$, as predicted by the theory that supposes inactivation proceeds independently of channel opening. Clearly, in Fig. 2*a*, the amount of inactivation observed with and without the delay in openings is the same.

The first hypothesis—that inactivation proceeds only, or primarily, from the open state—predicts that $P(t)$ and conditional probability function $w(T, t)$ should have the same amplitude and general form, but that the conditional probability

function should be shifted by 4.55 ms. In Fig. 2*b* the conditional probability function has been shifted to the left by 4.55 ms and superimposed on the overall opening probability function. The tail (that is, the falling phase) of the shifted conditional probability function $w(T, t + T)$ always lies below $P(t)$, contrary to what is expected if inactivation proceeds only from the open state.

The precise form of the first hypothesis (inactivation coupled to opening) we have tested is that $w(T, t + T) = P(t)$. The requirement that $w(T, t + T) = P(t)$ for values of t slightly greater than 0 tacitly assumes that the channel can exist in only one distinct closed state. The expected existence of multiple closed states is revealed, however, by changed behaviour of those channels whose opening is delayed: channels that have not opened during the first 4.55 ms have a significantly narrower distribution of times until first opening than does the entire channel population. Consequently, the conditional probability function $w(T, t)$ rises more rapidly to a peak value than does $P(t)$. This difference is not, however, large enough to produce the discrepancies in the tails of the two functions illustrated in Fig. 2*b*; nor can brief and undetected openings during the first 4.55 ms simply account for the observed departures seen in Fig. 2*b* from predictions of the first hypothesis.

Although our results are consistent with the hypothesis that inactivation proceeds independently of the activation processes, they may also be compatible with theories assuming inactivation is coupled to an activation step which precedes channel opening. If our conclusion has general applicability, however, theories which assume the inactivated state can be entered only, or predominantly, from an open state must be re-evaluated.

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1. Hodgkin, A. L. & Huxley, A. F. *J. Physiol., Lond.* **117**, 500–544 (1952).
2. Chandler, W. K., Hodgkin, A. L. & Meves, H. J. *Physiol., Lond.* **180**, 821–836 (1965).
3. Gillespie, J. I. & Meves, H. J. *Physiol., Lond.* **299**, 289–307 (1980).
4. Moore, L. E. & Jakobsson, E. *J. theor. Biol.* **33**, 77–89 (1971).
5. Bezanilla, F. & Armstrong, C. M. *J. gen. Physiol.* **70**, 549–566 (1977).
6. Goldman, L. & Schauf, C. L. *J. gen. Physiol.* **61**, 361–384 (1973).
7. Armstrong, C. M. & Bezanilla, F. *J. gen. Physiol.* **70**, 567–590 (1977).
8. Nonner, W. *J. Physiol., Lond.* **299**, 573–603 (1980).
9. Bean B. P. *Biophys. J.* (in the press).
10. Sigworth, F. J. & Neher, E. *Nature* **287**, 447–449 (1980).
11. Horn, R. & Patlak, J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6930–6934 (1980).

K⁺ channels close more slowly in the presence of external K⁺ and Rb⁺

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The voltage-sensitive gates regulating the permeability of ionic channels in nerve membrane were originally thought to be insensitive to the concentration¹ and species^{2,3} of readily permeant cations. In contrast, blocking cations—tetraethylammonium⁴, *N*-methylstrychnine⁵ or pancuronium⁶—when present in the inner end of potassium or sodium channels, hinder closing of the gates by a 'foot in the door' effect. Recent work on acetylcholine channels has clearly shown that the lifetime of the conducting state is prolonged by certain species of permeant cations^{7,8}. In this case, the presence of a permeant ion near the inner end of the channel is thought to prevent closing. Somewhat similar effects have recently been reported for the K⁺ channels of nerve membrane^{9–11}. We report here unequivocal evidence that the permeant cations K⁺ and Rb⁺, when present externally, slow the closing of K⁺ channels in squid axon, supporting the view that gating is indeed sensitive to the presence and species of permeant cations in the channels.

Experiments were performed on internally perfused squid giant axons, using standard axial wire voltage-clamp techniques¹². The composition of the internal solution in all

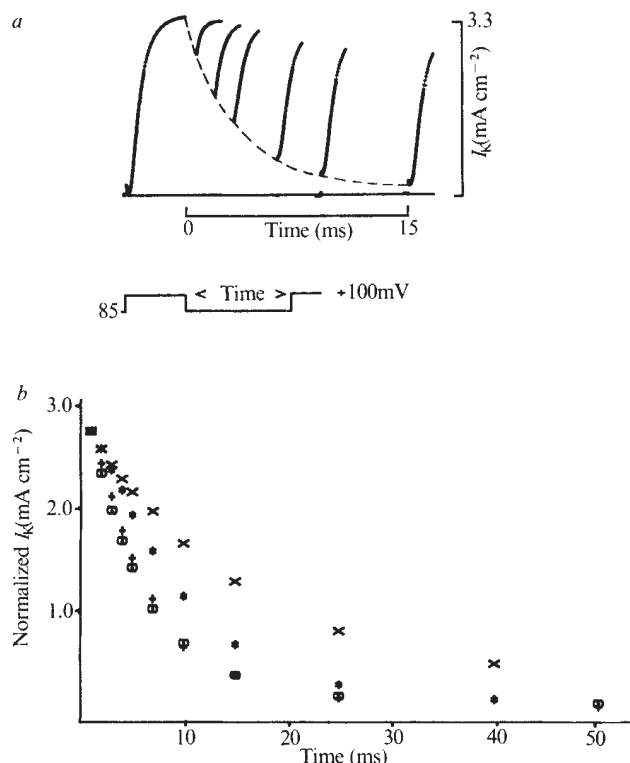


Fig. 1 Slow closing of K^+ channels in the presence of Rb^+ and K^+ . *a*, Potassium currents recorded from a tetrodotoxin-poisoned squid axon using the double-pulse procedure described in the text. The dashed curve gives the time course of decay of the potassium conductance. *b*, The decay of K^+ current at -85 mV is plotted in three external solutions. The data were taken from experiments like that shown in *a*, and solutions were applied in the order listed. I_K amplitude at the end of the first pulse in the four solutions was, in order, 3.4, 3.8, 2.8 and 4.0 mA cm^{-2} . The plots have been normalized for ease of comparison. Capacity and leakage currents were subtracted electronically. All external solutions contained 2×10^{-7} M tetrodotoxin and their compositions were as follows: $\square$, 440 Na^+ : 440 mM NaCl , 50 mM CaCl_2 , 10 mM Tris-Cl (Trizma 7.0). $*$, 100 K^+ , 340 Na^+ : 100 mM KCl , 340 mM NaCl , 50 mM CaCl_2 , 10 mM Tris-Cl . $\times$, 100 Rb^+ , 340 Na^+ : 100 mM RbCl , 340 mM NaCl , 50 mM CaCl_2 , 10 mM Tris-Cl . $+$, 440 mM Na^+ . Axon JL260A, 8°C .

experiments was 275 mM K^+ , 50 mM F^- , 225 mM glutamate , 10 mM Tris (Trizma 7.0) and 420 mM sucrose , $\text{pH } 7.0$. Compositions of the external solutions are given in Figs 1 and 2 legends. All experiments were performed at 8°C .

The pulse protocol and associated K^+ currents used for measuring the rate of decay of potassium conductance (g_K) are shown in Fig. 1*a*. The K^+ channels were activated by a first pulse from the holding potential (usually -85 mV) to $+100 \text{ mV}$ for 5–12 ms. The potential was then restored to the holding potential for a variable interval and g_K at the end of this interval was assayed with a second pulse to $+100 \text{ mV}$. Remaining g_K is proportional to the current at the beginning of the second step. Figure 1*a* illustrates the K^+ currents determined using this protocol for interpulse intervals 1–15 ms. The initial current during the second pulse becomes progressively smaller as the interval is lengthened, until after 15 ms nearly all the channels were closed. This relatively complicated protocol was used rather than simply measuring decay of the tail currents seen on return to the holding potential, because tail current kinetics can be seriously affected by depletion of ions in the confined space near the membrane¹³.

Decay of the potassium conductance as determined by this protocol is plotted in Fig. 1*b*. The membrane voltage was -85 mV , and the axon was bathed successively in 440 mM Na^+ , 100 mM K^+ + 340 mM Na^+ , 100 mM Rb^+ + 340 mM Na^+ , with a final determination in 440 mM Na^+ to test reversibility. The cor-

responding decay halftimes of the conductance were 5.2, 8.8, 14.8 and 5.6 ms. Relative to full Na^+ medium, equimolar substitution of 100 mM K^+ for Na^+ slows closing by a factor of 1.7, and 100 mM Rb^+ slows by a factor of 2.9. These effects were observed routinely, and were always reversible. This apparent stabilization of the open configuration of the channel may be visualized in terms of the foot in the door analogy, Rb^+ or K^+ playing the part of the foot. Closing of the channel is hindered or prevented by the presence of these ions at some locus in the channel.

We believe that Rb^+ slows closing more effectively than does K^+ because it has a longer dwell time in the channel, as indicated in Fig. 2, which contains plots of the instantaneous current-voltage curve for a fibre immersed in 440 mM Na^+ , 200 mM K^+ + 240 mM Na^+ , 200 mM Rb^+ + 240 mM Na^+ , and again in 440 mM Na^+ . As indicated in the inset, the K^+ channels were activated by a 4-ms pulse to 100 mV , and the potential was then stepped to the values given on the abscissa. Current was measured $\sim 60 \mu\text{s}$ after the second step. With 200 mM K^+ the plot is nearly a straight line with a zero current intercept at $+6 \text{ mV}$. This is somewhat more positive than the theoretical value predicted from internal and external K^+ concentrations because of K^+ ion accumulation in the Schwann cell space during the activating pulse. With 200 mM Rb^+ , the zero current intercept is almost the same ($+2 \text{ mV}$), but the plot has a pronounced curvature at negative potentials, showing that Rb^+ ions carry current inwards less efficiently than do K^+ ions. At large positive potentials the outward current in the presence of the two cations

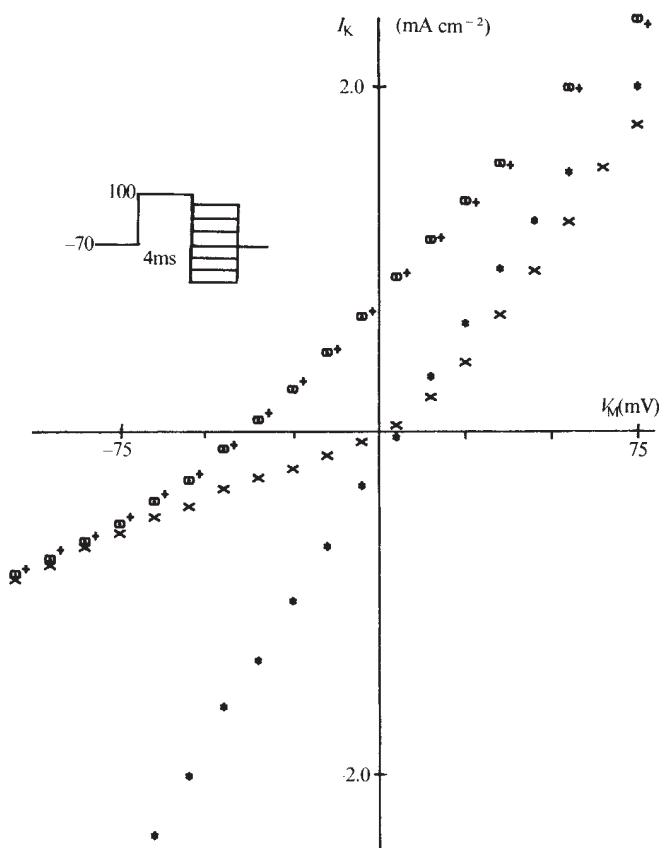


Fig. 2 Instantaneous current-voltage relations of activated K^+ channels in four solutions. Channels were activated by a pulse to $+100 \text{ mV}$ for 4 ms (inset), and voltage was then changed to the value given on the abscissa. Current immediately following this second voltage step was measured and is plotted as a function of voltage in the second step. Few channels can open or close in the short time before the measurement, so the curve is proportional to the I - V relationship of a single open K^+ channel. The curves were determined in three solutions in the order listed. Symbols and solutions are as described in Fig. 1 legend, except that 200 mM rather than 100 mM Rb^+ or K^+ is substituted for an equal amount of Na^+ . Axon JL280A, 8°C .

is nearly the same and the two curves appear to be converging. The outward current is carried by K^+ ions from the internal medium in all four plots.

The similarity of the reversal potential in both solutions indicates that Rb^+ and K^+ enter the channels equally well¹⁴. However, the relatively small inward current in 200 mM Rb^+ must mean that once Rb^+ is in the channel it is less mobile than K^+ and thus has a longer dwell time in the channel.

Turning again to the foot in the door, we postulate that Rb^+ slows closing more because it behaves as a stickier foot. Clearly this postulate must be tested by experiments with other permeant ions.

It is of interest to speculate where K^+ and Rb^+ are located in the channel when they exercise their gate-slowing action. There are several reasons for thinking the site is near the inner end of the channel. First, blocking cations such as tetraethylammonium are in this location when they hinder gate closing. Barium, in contrast, occupies a site near the outer end of the channel, and seems to have the opposite effect: it stabilizes the closed configuration of the channel¹⁵. Finally, the gate-slowing locus in acetylcholine channels is thought to be near the inner end of the channel. We hope that further examination of these questions will give a better picture of the gating machinery of K^+ channels.

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- Hodgkin, A. L. & Huxley, A. F. *J. Physiol., Lond.* **116**, 449–472 (1952).
- Chandler, W. K. & Meves, H. *J. Physiol., Lond.* **180**, 788–820 (1965).
- Hille, B. *J. gen. Physiol.* **58**, 599–619 (1971).
- Armstrong, C. M. *J. gen. Physiol.* **58**, 413–437 (1971).
- Cahalan, M. D. & Almers, W. *Biophys. J.* **27**, 57–74 (1979).
- Yeh, J. Z. & Armstrong, C. M. *Nature* **273**, 387–389 (1978).
- Ascher, P., Marty, A. & Neild, T. O. *J. Physiol., Lond.* **278**, 177–206 (1978).
- Marchais, D. & Marty, A. *J. Physiol., Lond.* **297**, 9–45 (1979).
- Arhem, P. *Acta physiol. scand.* **108**, 7–16 (1980).
- Dubois, J. M. & Bergman, C. *Pflügers Arch. ges. Physiol.* **370**, 185–194 (1977).
- Stühmer, W. & Conti, F. in *A. Meet. Deutsche Gesellschaft Biophysik* (eds Adam, G. & Stark, G.) (Springer, New York, 1979).
- Bezanilla, F. & Armstrong, C. M. *J. gen. Physiol.* **70**, 549–566 (1977).
- Frankenhauser, B. & Hodgkin, A. L. *J. Physiol., Lond.* **131**, 341–376 (1956).
- Hille, B. in *Membranes. A Series of Advances* Vol. 3 (ed. Eisenman, G.) (Dekker, New York, 1975).
- Armstrong, C. M. & Taylor, S. R. *Biophys. J.* **30**, 473–488 (1980).

Transcription of single-copy vitellogenin gene of *Xenopus* involves expression of middle repetitive DNA

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The genome of eukaryotes consists of single-copy DNA interspersed with middle repetitive DNA. Both types of DNA are known to be transcribed (reviewed in ref. 1). Several reports have shown that some nuclear RNA is composed of single-copy DNA transcripts covalently linked to sequences transcribed from repetitive DNA^{2–4}, but the significance of these nuclear transcripts is unknown; there is no convincing evidence that these nuclear transcripts containing repetitive DNA sequences are precursors to mRNAs. To elucidate this question we have analysed the structure of the two related vitellogenin genes *A1* and *A2* of *Xenopus laevis*^{5,6}. The expression of these genes is initiated in liver cells by oestrogen (reviewed in refs 7–9). Preliminary experiments demonstrated that the flanking region as well as several introns of these genes contain middle repetitive DNA⁹. We have now identified and mapped six different repetitive DNA sequences within the transcribed introns of the *A1* vitellogenin gene. Further characterization of these sequences implies that they are evolutionary unstable. Analysis of nuclear RNA revealed that transcripts of the six sequences are also found in non-vitellogenic liver cells, suggesting that these repetitive DNAs are also present in transcriptional units active in the absence of oestrogen.

To identify and locate the repetitive DNA found in the *A1* vitellogenin gene we constructed subclones of the seven largest introns of the *A1* vitellogenin gene (Fig. 1). This was achieved by insertion of 400–600-base pair fragments derived from the cloned *A1* gene into the plasmid pBR322. Intron-specific clones were selected by the lack of hybridization to *A2* vitellogenin genomic clones, which are homologous in the exon sequences but differ in sequence in analogous introns⁵. To locate the origin of these intron specific subclones the labelled subclones were hybridized to blots containing genomic clones restricted with various enzymes (data not shown). In most cases this approach was sufficient to identify the intron from which the subclones were derived. The subclones were numbered according to the intron of origin and independent subclones from the same intron were distinguished by a letter. In cases where the subclones could not be mapped unambiguously we hybridized the insert of the subclone to an R-loop formed between a genomic clone and the vitellogenin mRNA. Figure 1 gives the position of 18 independent subclones derived from the 7 largest of the 33 introns. All subclones map to a unique position within *A1*, establishing that they are not repeated in other introns of the gene and they are also absent from the *A2* vitellogenin gene.

To monitor repetitive DNA sequences in the introns the subcloned DNAs were separated on an agarose gel and transferred to nitrocellulose filters. The blots were hybridized with ³²P-labelled total *X. laevis* DNA and the hybridized DNA was visualized by autoradiography (Fig. 2). In these hybridization conditions DNA fragments containing repetitive DNA are detected, whereas single-copy DNA does not react^{9–11}. Figure 2 demonstrates that repetitive DNA sequences are present in six of the seven introns tested (see Fig. 1 for locations). As a control a slot with an *A1*-specific vitellogenin cDNA clone (pXlvc23) was included to verify that single-copy DNA did not react. The various intensities of the hybridizations suggest different repetition frequencies for the six sequences within the *Xenopus* genome. This conclusion could be confirmed by hybridizing a large excess of *Xenopus* DNA to small amounts of labelled inserts isolated from the subclones. This *C₀t* analysis revealed that the repetitive sequences are repeated a few hundred to a few thousand times in the genome (data not shown).

Characterization of genomic DNA has shown that in *Xenopus* as in most higher eukaryotes middle repetitive sequences are interspersed with single-copy DNA segments 1–2 kilobases long¹². Our data demonstrate that a similar pattern of interspersion is also present within a specific gene, because the unusually large *A1* vitellogenin gene of 21 kilobases is interrupted by at least six different repetitive sequences.

To analyse the distribution of the repetitive sequences found in the *A1* vitellogenin gene throughout the *Xenopus* genome, we selected three subclones for further analysis. We isolated these cloned inserts and hybridized the labelled DNA fragments to a blot containing total *Xenopus* DNA restricted by *Eco*RI. The autoradiogram given in Fig. 3 reveals that the repetitive DNA elements tested are distributed over numerous *Eco*RI restriction fragments of different size, suggesting that the repetitive DNAs are not clustered.

Our earlier results have suggested that the *A1* and *A2* vitellogenin genes are the product of a duplication of an ancestral gene⁵. As the six repetitive sequences identified within the *A1* gene are absent in the *A2* gene, we assume that the repetitive sequences have been introduced into the *A1* gene quite recently during evolution or have diverged rapidly in the two closely related genes. To obtain further insight into the evolutionary stability of the six repetitive sequences, we hybridized a blot with all 18 intron subclones with ³²P-labelled total DNA from the related species *Xenopus tropicalis*. As Fig. 2 demonstrates, the repetitive sequences found in introns 13, 18 and 23 of the *A1* vitellogenin gene of *X. laevis* are not detected as repetitive DNA in *X. tropicalis*, suggesting that these sequences are evolutionarily unstable. In this context it is of interest that *X. laevis* cDNA clones of the vitellogenin genes cross-react with genomic DNA of *X. tropicalis* with a specificity

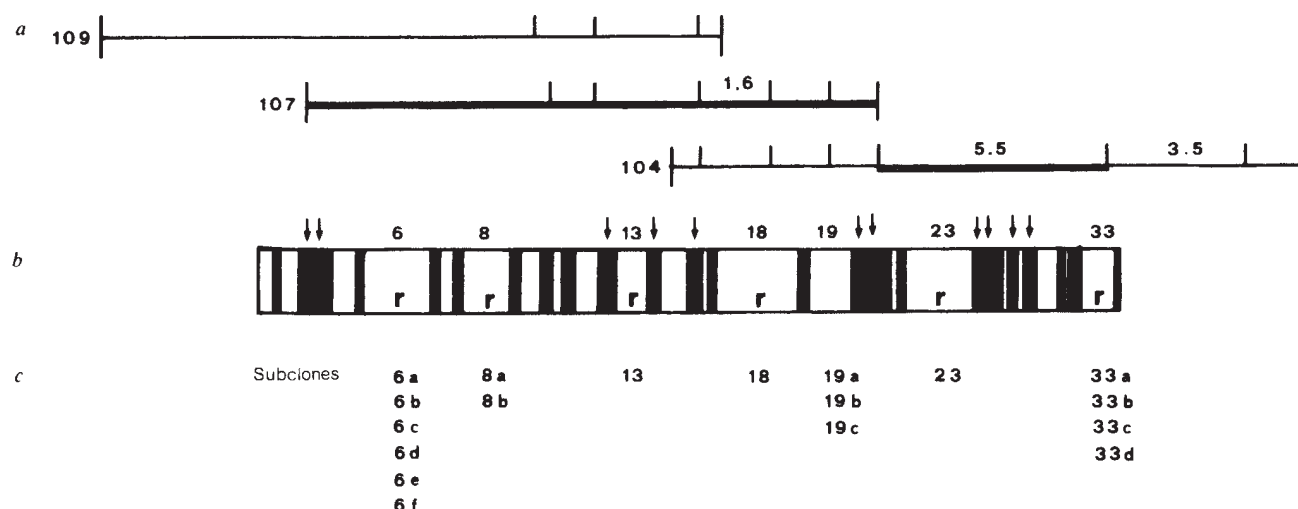


Fig. 1 Construction and location of various intron-specific subclones of the A1 vitellogenin gene. *a*, Genomic vitellogenin gene A1 clones with their *Eco*RI sites^{5,9}. The thick line represents the restriction fragments used for subcloning and the sizes are given in kilobases. *b*, The A1 vitellogenin gene with exons (black boxes) and introns (white boxes) is aligned to the genomic clones. The subcloned introns are numbered and small introns are indicated by arrows⁵. The letter *r* in introns denotes repetitive DNA elements identified in the present work. *c*, Location of the intron subclones obtained. The genomic clone λ Xlv107 and the 5.5-kilobase *Eco*RI fragment of the genomic clone λ Xlv104 were partially digested with *Alu*I and *Hae*III, fragments of 400–600 base pairs were isolated electrophoretically¹⁷ and cloned into the *Pst*I site of pBR322 by G·C tailing²². Tetracycline-resistant, ampicillin-sensitive clones were screened by hybridization with A1 and A2 vitellogenin genomic clones²³. Clones hybridizing with A1 but not with A2 were isolated and again tested for lack of hybridization with the A2 genomic clone λ Xlv128, containing the complete A2 gene⁵. Further details and the methods used for mapping the position of the subclones are given in the text. Subclone 18 was prepared by *Hha*I digestion of the isolated 1.6-kilobase *Eco*RI fragment of the genomic clone λ Xlv107 and inserting a 1-kilobase *Hha*I-*Eco*RI fragment into the *Pst*I site of pBR322 by G·C tailing. This clone also failed to react with the A2 genomic clone λ Xlv 128.

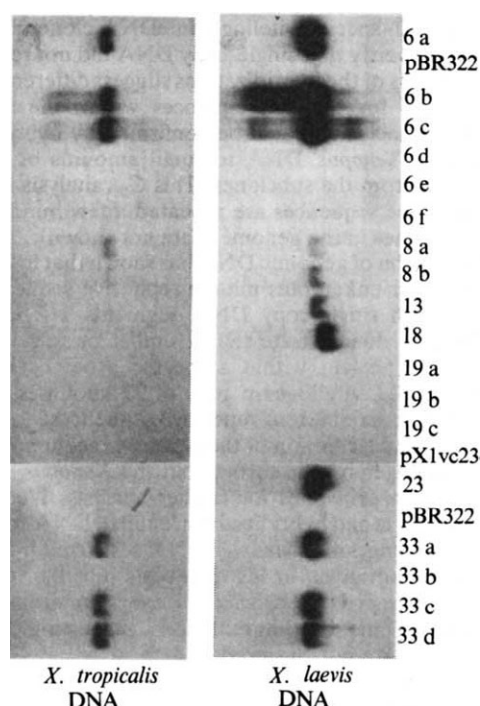


Fig. 2 Identification of repetitive sequences within the subclones. The intron subclones, the vector pBR322 and the A1 vitellogenin cDNA clone pXlvc23¹⁶ were digested with *Eco*RI, separated electrophoretically on a 1% agarose gel and transferred to nitrocellulose filters. The blot was hybridized⁹ at 37 °C for 72 h with *X. laevis* or *X. tropicalis* DNA labelled by nick translation, washed and exposed for autoradiography as described elsewhere⁹. Electrophoresis is from right to left.

suggesting that these sequences differ by about 10% (R. Jaggi, personal communication). In complementary experiments we hybridized labelled inserts of intron subclones 6, 13 and 18 to blots of *Eco*RI-digested genomic DNA. The results included in Fig. 3 confirm and extend the above conclusions. The

repetitive DNA of intron 6 is present in *X. tropicalis* and is distributed over many *Eco*RI restriction fragments whereas the repetitive DNAs of the intron subclones 13 and 18 do not cross-hybridize with *X. tropicalis* DNA, suggesting that these sequences are even absent as single-copy DNA.

Analysis of nuclear RNAs containing vitellogenin mRNA sequences has shown directly that the large introns 11–33 of the vitellogenin gene are transcribed as part of a large polyadenylated precursor RNA¹³. Assuming that all the other introns are also transcribed, we conclude that the transcription of the single-copy A1 vitellogenin gene involves the expression of a specific set of at least six repetitive DNA sequences located in introns. To answer the question of whether these repetitive sequences are also present in other transcriptional units, we analysed different nuclear RNA preparations for transcripts complementary to the 18 subclones. First, we hybridized a blot of all 18 subclones with a labelled polyadenylated nuclear RNA enriched for the vitellogenin precursor RNA. As Fig. 4 illustrates, all intron subclones hybridize with this RNA preparation, which agrees with the assumption that indeed all introns are transcribed as part of the primary transcript. If we used total polyadenylated nuclear RNA from an oestrogen-treated female producing large amounts of vitellogenin, some subcloned intron sequences could hardly be detected in these nuclear RNAs, indicating that these sequences are too dilute in this RNA preparation (Fig. 4). To elucidate whether the repetitive sequences found in the A1 vitellogenin gene are also part of other transcriptional units, we used labelled polyadenylated nuclear RNA from the liver of an unstimulated male, known not to produce any vitellogenin mRNA^{9,14,15}. Control experiments included in Fig. 4 confirm the absence of vitellogenin gene transcripts as shown by the failure of this RNA to hybridize with the cloned vitellogenin cDNA pXlvc23. On the other hand, the data given in Fig. 4 clearly establish that this RNA preparation contains sequences hybridizing to all six repetitive sequences found in the A1 vitellogenin gene. This implies that repetitive sequences of A1 are also found in transcriptional units active even in the absence of oestrogen.

Our analysis of the A1 vitellogenin gene of *X. laevis* leads to the following main observations on transcribed middle repetitive DNA sequences.

As the six repetitive DNA sequences found in the *A1* gene are absent from the closely related *A2* gene, and as these two genes are apparently under coordinate control by oestrogen^{16,17}, one is tempted to conclude that these repetitive sequences have no function for gene expression.

The repetitive sequences of the *A1* gene have certain characteristics of mobile genetic elements. Assuming that genes *A1* and *A2* have arisen by gene duplication, the dramatic change in sequence and length of analogous introns⁵ suggests that these sequences may have evolved to a large degree by insertions and deletions. Apparently, the inserted or deleted DNA sequences are frequently repetitive DNA. This holds also for the *A1* vitellogenin gene variant which contains a repetitive sequence in the intron 11 (ref. 9).

Comparison of the repetitive DNAs of the *A1* vitellogenin gene with those of the related species *X. tropicalis* provides additional evidence for the instability of these sequences. In fact, three out of the six repetitive DNAs are absent from the DNA of *X. tropicalis*, which represents the most primitive type of *Xenopus*¹⁸. All these findings suggest that the repetitive sequences within the *A1* genes are unstable elements.

The finding that the repetitive sequences in the oestrogen-inducible *A1* gene are also present in transcriptional units active in the absence of oestrogen (Fig. 4) argues against a simple model in which the six intron-specific repetitive sequences are regulatory elements involved in oestrogen-inducible gene expressions. Future experiments may reveal that repetitive sequences are common in many transcribed introns. In fact, repetitive sequences have recently been detected within introns of the conalbumin gene¹¹ and the ovalbumin-like *X* gene¹⁹ of the chicken. Because different cells express specific classes of genes, a widespread occurrence of repetitive DNA within transcriptional units would explain the well-documented fact that different tissues contain characteristic sets of repetitive DNA transcripts (reviewed in ref. 1).

All our data on the repetitive DNA within the *A1* gene imply that these sequences reside in this gene without having any obvious function. They might represent some kind of selfish or parasitic DNA^{20,21}. However, until further analyses are per-

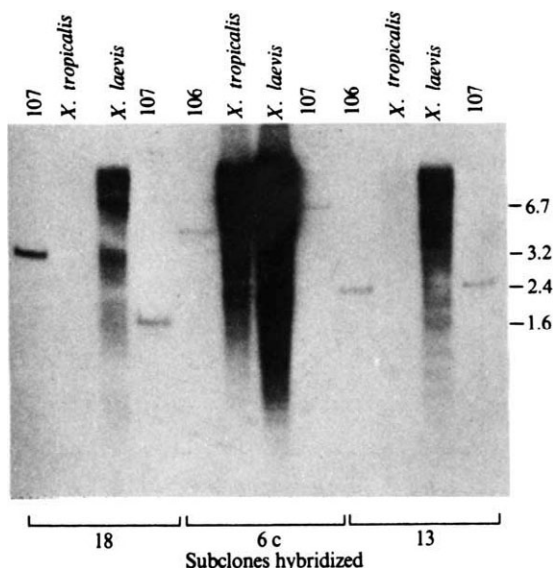


Fig. 3 Distribution of the intron-specific repetitive sequences throughout the genome. Aliquots (10 μ g) of *X. laevis* and *X. tropicalis* DNA were restricted with *Eco*RI, separated on a 1% agarose gel, transferred to nitrocellulose filters and hybridized with 32 P-labelled inserts of the subclones 6c, 13 and 18. The inserts were cut out by *Pst*I digestion and isolated electrophoretically as described elsewhere¹⁷. Hybridization was as in Fig. 2. Genomic clones λ Xlv106 and λ Xlv107⁵ were included as markers in single copy amounts and the molecular weight of these markers are given in kilobases.

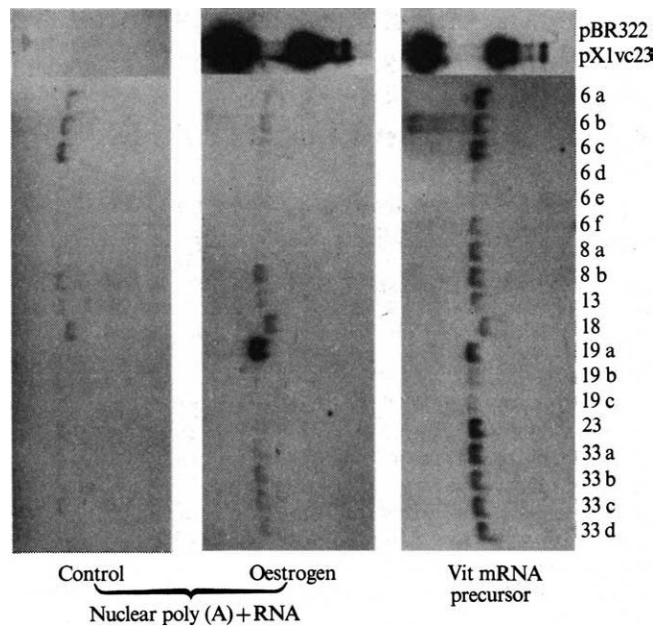


Fig. 4 Transcripts of intron-specific repetitive DNA sequences. Similar blots with intron subclones as in Fig. 2 were hybridized with nuclear polyadenylated liver RNA enriched for vitellogenin mRNA precursor¹³, nuclear polyadenylated liver RNA from an oestrogen-treated female¹³ and nuclear polyadenylated liver RNA from control males not stimulated with oestrogen. The RNAs were labelled *in vitro* to a specific radioactivity of $2-4 \times 10^7$ c.p.m. per μ g as follows: 2 μ g RNA was partially hydrolysed for 30 min in 0.2 M NaOH at 0°C (ref. 24), neutralized with HCl, adjusted in a final volume of 50 μ l to 120 mM Tris-HCl (pH 7.6), 10 mM MgCl₂ and 5 mM dithiothreitol, incubated with 50 μ M [γ -³²P]ATP (Amersham, PB 10168) and 5 units T₄ polynucleotide kinase (Boehringer) for 30 min at 37°C and purified on a small Sephadex G-50 column. Hybridization was as in Fig. 2. If the RNA was hydrolysed in control experiments with 0.5 M NaOH before hybridization, no positive reactions were observed, excluding the possibility that the hybridization is due to contaminating DNA in our RNA preparation.

formed, it remains possible that these repetitive sequences or at least some of them, are essential for gene regulation according to the model proposed by Britten and Davidson¹.

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1. Davidson, E. H. & Britten, R. J. *Science* **204**, 1052-1059 (1979).
2. Fedoroff, N., Wellauer, P. K. & Wall, R. L. *Cell* **10**, 597-610 (1977).
3. Scheller, R. H., Constantini, F. D., Kozlowski, M. R., Britten, R. J. & Davidson, E. H. *Cell* **15**, 189-203 (1978).
4. Constantini, F. D., Britten, R. J. & Davidson, E. H. *Nature* **287**, 111-117 (1980).
5. Wahli, W., Dawid, I. B., Wyler, T., Weber, R. & Ryffel, G. U. *Cell* **20**, 107-117 (1980).
6. Wahli, W. & Dawid, I. B. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1437-1441 (1980).
7. Tata, J. R. & Smith, D. F. *Recent Prog. Horm. Res.* **35**, 47-95 (1979).
8. Shapiro, D. J. & Baker, H. J. in *Ontogeny of Receptors and Reproductive Hormone Action* (eds Hamilton, T. H., Clark, J. H. & Sadler, W. A.) 309-330 (Raven, New York, 1979).
9. Wahli, W., Dawid, I. B., Ryffel, G. U. & Weber, R. *Science* (in the press).
10. Fritsch, E. F., Lawn, R. M. & Maniatis, T. *Cell* **19**, 959-972 (1980).
11. Cochet, M. *et al. Nature* **288**, 567-574 (1979).
12. Davidson, E. H. & Britten, R. J. *Q. Rev. Biol.* **48**, 565-609 (1973).
13. Ryffel, G. U., Wyler, T., Muellener, D. B. & Weber, R. *Cell* **19**, 53-61 (1980).
14. Ryffel, G. U., Wahli, W. & Weber, R. *Cell* **11**, 213-221 (1977).
15. Baker, H. J. & Shapiro, D. J. *J. biol. Chem.* **252**, 8428-8434 (1977).
16. Wahli, W. *et al. Cell* **16**, 535-549 (1979).
17. Felber, B. K. *et al. Eur. J. Biochem.* **105**, 17-24 (1981).
18. Bisbee, C. A., Baker, M. A., Wilson, A. C., Hadzi-Azimi, J. & Fischberg, M. *Science* **195**, 785-787 (1977).
19. Heilig, R., Perrin, F., Gannon, F., Mandel, J. L. & Chambon, P. *Cell* **20**, 625-637 (1980).
20. Doolittle, W. F. & Sapienza, C. *Nature* **284**, 601-603 (1980).
21. Orgel, L. E. & Crick, F. H. C. *Nature* **284**, 604-607 (1980).
22. Wieringa, B. *et al. Nucleic Acids Res.* **7**, 2147-2163 (1979).
23. Grunstein, M. & Hogness, D. S. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3961-3965 (1975).
24. Wahli, W., Wyler, T., Weber, R. & Ryffel, G. U. *Eur. J. Biochem.* **86**, 225-234 (1978).

Isolation of virus from brain after immunosuppression of mice with latent herpes simplex

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Herpes simplex virus (HSV) is usually present in a latent form in the trigeminal ganglion of man¹⁻³. Various stress factors may induce virus reactivation, which is manifest by a lip lesion (innervated from the trigeminal ganglion) and the production of infectious virus. The considerable experimental efforts to define the conditions that lead to the reactivation of latent HSV have concentrated on isolating virus either from the original extraneural site of virus inoculation, or from cell-free homogenates of sensory ganglia from latently infected animals⁴⁻¹⁵. Recent DNA hybridization experiments resulted in the demonstration of the presence of HSV genomes in the brain tissue of both latently infected mice, and of humans who showed no clinical symptoms of HSV (ref. 16 and N. Fraser, personal communication). This led us to consider the possibility that HSV may be present in brain tissue as the result of either reactivation of the virus in brain cells or the passage of reactivated virus from trigeminal ganglia through the brain stem to the brain. The presence of infectious HSV in brain tissue has not previously been demonstrated; yet this could be a factor in chronic, relapsing neurological diseases such as multiple sclerosis. We have now shown experimentally that mice carrying latent HSV in their trigeminal ganglia may, following massive immunosuppression, express infectious virus in the central nervous system (CNS).

Male C3H mice 10-12 weeks of age were lip-inoculated with HSV type 1 (HSV-1) (1×10^7 plaque-forming units (PFU) of laboratory strain 2) as previously described¹⁰. Four weeks later, surviving animals were divided into two groups (1 and 2) of 20 animals each. Groups 1 and 3 (the latter an uninfected control group of 20 animals) were thymectomized, and group 2 was sham thymectomized. All three groups were irradiated (850 rad) 8 weeks later, and reconstituted with $1-2 \times 10^7$ C3H bone marrow cells that had been treated with anti-Thy-1.2 antibody and complement ('B' mice). Groups 1 and 3 also received intraperitoneally (i.p.) 0.5 ml of a 1:10 dilution of antithymocyte serum (ATS) on days 4, 6 and 8 post-irradiation. Animals in this series were followed clinically, and serial serum samples were obtained for analysis of anti-HSV antibodies measured by solid phase radioimmunoassay (RIA), with HSV-infected CV₁ (indicator) cells as the immunoabsorbent.

The RIA was done as described elsewhere¹⁷. Briefly, appropriate dilutions of antisera were incubated with immunoabsorbent (in 96-well flat-bottomed tissue culture plates in which 26-h post-infection confluent monolayers of CV₁ cells were fixed with 0.15% glutaraldehyde). After 90 min of incubation at room temperature, the plates were washed and ¹²⁵I-labelled rabbit anti-mouse F(ab')₂ antibody was added. After the final incubation and washes, each well was solubilized in detergent, and the amount of antibody bound determined by γ counting.

Animals in group 1 became lethargic and ataxic between days 5 and 14 post-irradiation whereas those in groups 2 and 3 remained healthy. However, all mice in group 1 recovered and when the study was terminated on day 57, their mortality rate was the same as that for group 3. The animals were bled from the retro-orbital sinus at various times after irradiation and their

sera fractionated for determination by RIA of the presence of anti-HSV antibodies in various clones of immunoglobulins. Results of serial anti-HSV antibody determinations are shown in Fig. 1. The major difference between groups 1 and 2 was the level of anti-HSV antibody circulating at the end of the study. Antibody was detected in sera from group 2 but not from group 1 animals on day 57.

Fragments of trigeminal ganglia, lip mucosa and brain tissue were minced and co-cultivated with CV₁ cells. If a cytopathic effect (CPE) was observed in the culture of indicator cells, the virus was passed and identified in a neutralization test by inhibition of the CPE by anti-HSV immune rabbit serum. At the end of the experiment (day 57), virus was isolated from the trigeminal ganglia of 91% of group 1 and 93% of group 2 survivors but not from the trigeminal ganglia of group 3 mice. Virus was not recovered from the brains of mice from any group.

The next step was to investigate whether HSV could be isolated from the brain tissue of animals when they became acutely ill. We therefore infected a second series of mice (30 per group) with HSV and (after irradiation and immunosuppression) treated them as in the first experiment, the only difference being that animals were killed for sampling on selected days after irradiation (Tables 1, 2). HSV was isolated from the trigeminal ganglia of most of the mice that had been latently infected with HSV (groups 1 and 2, Table 1). Virus was present in the brain tissue in 50% of the mice from group 1 sampled on days 4 and 7 (Table 1), but not in the brain tissue of any other animals. Recrudescence of virus in the CNS thus seems to be dependent on massive immunosuppression, and is accompanied by the development of transient, but not fatal, neurological symptoms. It could be argued that reactivation of HSV might have occurred in tissue culture rather than *in vivo*; however, this is only remotely possible as on many occasions cultivation of CNS tissue containing the viral genome did not result in the isolation of infectious HSV (N. Fraser, personal communication).

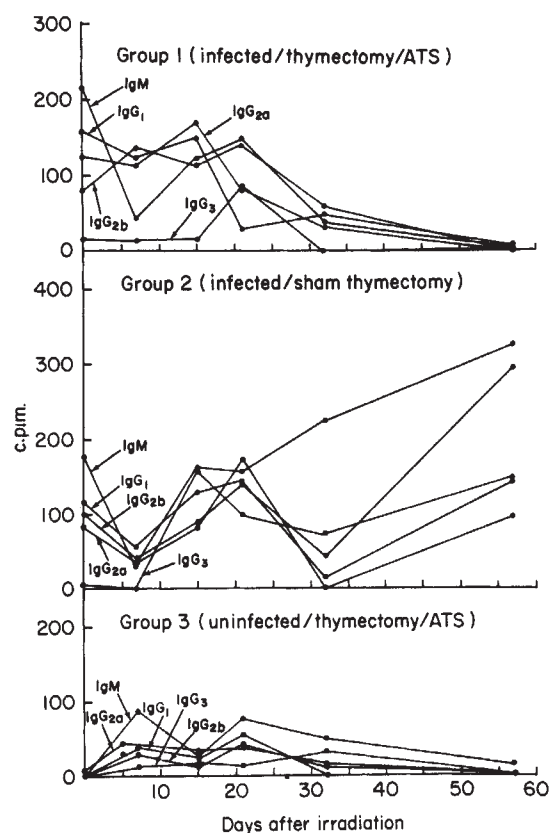


Fig. 1 Levels of serum antibody in latently infected and normal mice that were irradiated (850 rad) and reconstituted with bone marrow 8 weeks after thymectomy or sham thymectomy. Mice in groups 1 and 2 were also dosed with antithymocyte serum (ATS) 4, 6 and 8 days after irradiation.

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Table 1 Recovery of virus from the brains of mice latently infected with HSV after severe immunosuppression

Group	Treatment				Days after 850 rad	No. of mice sampled	Virus isolation*			
	Latent HSV	ATx	850 rad	ATS			Lip	Trigeminal ganglia	Brain	Negative
1	+	+	+	+	4	8	0	8	4	0
	+	+	+	+	7	8	1	8	5	0
	+	+	+	+	10	7	1	4	0	2
2	+	—	+	—	4	10	0	9	0	1
	+	—	+	—	7	10	1	8	0	1
	+	—	+	—	10	9	2	6	0	1
3	—	+	+	+	4	10	0	0	0	10
	—	+	+	+	7	10	0	0	0	10
	—	+	+	+	10	10	0	0	0	10

Male C3H mice were inoculated in the lip with 1.0×10^7 PFU of HSV (groups 1 and 2), thymectomized (ATx) 4 weeks later (groups 1 and 3), irradiated and reconstituted with bone marrow after a further 8 weeks (all groups) and injected with ATS 4, 6 and 8 days later.

* Tissues were minced and virus isolated by co-cultivation with CV₁ cells. The identity of the brain isolates was determined by neutralization. In all cases, if virus was isolated from the lip or brain, it was also isolated from the trigeminal ganglia.

Table 2 Persistence of antibody in thymectomized, 850-rad HSV-infected mice

Group	Day after irradiation	Level of serum antibody				
		IgM	IgG1	IgG2a	IgG2b	IgG3
1	4	228 ± 107	436 ± 125	417 ± 43	407 ± 173	162 ± 58
	7	192 ± 60	389 ± 135	401 ± 4	284 ± 49	121 ± 18
	10	398	403	263	166	258

The results are expressed, minus background, as mean c.p.m. ± s.d. The mice are those from group 1, Table 1.

The effect described above presumably reflects the removal of both 850 rad-sensitive and radiation-resistant (ATS-sensitive) memory T cells. Serum antibody levels were relatively high in the immunosuppressed mice when virus was isolated from the CNS (Tables 1, 2). The possibility thus exists that conditions (such as stress or intercurrent virus infections) that might cause a transient specific defect in HSV-specific cell-mediated immunity¹⁸ could result in the emergence of infectious virus in the CNS. This could then be controlled by non-T cell-dependent

mechanisms; virus was isolated from the brains of mice on day 7, but not on day 10 after immunosuppression. New T cells would not be expected to emerge from the thymus for 2–3 weeks. Furthermore, persistence of specific circulatory antibody of any class (Fig. 1) does not seem to be essential for preventing virus reactivation.

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- Bastian, F. O., Rabson, A. S., Yee, C. L. & Traika, T. S. *Science* **178**, 306–307 (1972).
- Baringer, J. R. & Swoveland, P. *New Engl. J. Med.* **288**, 648–650 (1973).
- Warren, K. G. *et al. Lancet* **ii**, 637–639 (1977).
- Anderson, W. A., Margruder, B. & Kilbourne, E. *Proc. Soc. exp. Biol. Med.* **107**, 628–632 (1961).
- Underwood, G. & Weed, S. *Infect. Immun.* **10**, 471–474 (1974).
- Blyth, W., Hill, J., Field, H. & Harbour, D. *J. gen. Virol.* **33**, 547–550 (1976).
- Hurd, J. & Robinson, R. *J. Antimicrob. Chemother.* **3**, 99–106 (1977).
- Hill, T., Blyth, W. & Harbour, D. *J. gen. Virol.* **39**, 21–28 (1978).
- Walz, M., Price, R. & Notkins, A. *Science* **184**, 1185–1187 (1974).
- Stevens, J., Cook, M. & Jordan, M. *Infect. Immun.* **11**, 635–639 (1975).
- Openshaw, H., Asher, L., Wohlenberg, C., Sekizawa, T. & Notkins, A. *J. gen. Virol.* **44**, 205–215 (1979).
- Hill, T., Field, H. & Blyth, W. *J. gen. Virol.* **28**, 341–353 (1975).
- Nesburn, A., Green, M., Radnoti, M. & Walker, B. *Infect. Immun.* **15**, 772–775 (1977).
- Price, R. & Schmitz, J. *Infect. Immun.* **19**, 523–532 (1978).
- Kurata, T., Kurata, K. & Aoyama, Y. *Jap. J. exp. Med.* **48**, 427–435 (1978).
- Sequiera, L. *et al. Lancet* **ii**, 609–612 (1979).
- Frankel, M. E. & Gerhard, W. *Molec. Immun.* **16**, 101–106 (1979).
- Howes, E., Taylor, W., Mitchison, N. & Simpson, E. *Nature* **277**, 67–68 (1979).

Disorientation of inexperienced young pigeons after transportation in total darkness

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Leask's proposal¹ that magnetic field detection in birds is accomplished by an optical double-resonance process in the rhodopsin molecules in the retina, a spatially well ordered cellular array, is intriguing. Being an axial rather than a polar phenomenon, it is consistent with observations that birds do not make use of the polarity of the magnetic field². The hypothesis nevertheless requires that light is necessary for sensing magnetic fields, which has prompted us to perform the homing experiments with pigeons reported here. It is known that transportation in a distorted magnetic field causes an increase in scatter, frequently even random, in the initial orientation of young pigeons^{3–6}, and we sought to discover whether transportation in total darkness would have a similar effect. The results are consistent with Leask's hypothesis but their explanation is not unambiguous. Plainly, however, transportation in total darkness, like the disruption of magnetic or olfactory^{3,7} information en route, is another way of preventing the collection of meaningful orientation information during the outward journey to the release site.

Because the effect of treatments during displacement is most pronounced in tests with very young birds without any previous training experience^{5,6}, we used only untrained birds younger than 12 weeks in the tests reported here. We chose a release site 65 km SSW of the home loft (home direction 17°). Depending on local traffic, the driving time was 50–75 min. On sunny days, the birds were released singly, alternating experimentals and controls. They were observed with 10 × 40 binoculars until they vanished from sight; their departure bearings were recorded to the nearest 5° and later statistically evaluated as described by Batschelet⁸. Homing times and homing success could not be evaluated because the distance of 65 km proved too large for our young birds, and very few returned.

Table 1 Effect of transportation in total darkness on orientation of pigeons after release

Test no.	n	Controls		Experimentals		
		α (deg)	r	n	α (deg)	r
1	9 ^s	6	0.81*	9	286	0.20‡
2	9 ^s	345	0.70†	10	272	0.14‡
3	9 ^l	11	0.76†	8	41	0.23‡
4	10 ^l	345	0.82*	10	138	0.29‡

With the controls, s indicates birds that sat in the dark for 30 min after arriving at the release site, and l indicates birds transported in the lighted box (see text). Significance by the Rayleigh test: *, $P < 0.001$; †, $P < 0.01$; ‡, $P > 0.05$, not significant.

To demonstrate the effect of transportation in an altered magnetic field, the birds were transported in wooden baskets, the walls and roof of which consisted of small wooden poles allowing the birds to see the outside. For the controls this basket was placed on the floor of the transportation vehicle, a Volkswagen bus with windows all round, where the magnetic field was only very slightly distorted ($<10\%$) by the metal frame of the car. The experimentals were transported in a second vehicle of the same type, but were placed in the centre of a pair of Helmholtz coils which produced a horizontal magnetic field of ~ 0.4 G pointing towards the front of the car. This field added to the local field as we described before^{5,6}, resulting in a strongly distorted total field whose characteristics depended on the direction of driving. After arriving at the release site, both baskets were placed on the ground, giving the birds access to the natural environmental stimuli. In four such tests the normally transported control birds were significantly homeward oriented, whereas the birds transported in the artificially altered magnetic field showed no significant directional tendency ($P < 0.01$, Mardia Watson Wheeler test with standardized mean directions). The results are summarized in Fig. 1.

To test whether transportation in total darkness produced a similar effect, we placed the basket of experimental birds in a light-tight wooden box, which was transported on the rooftop baggage rack of the vehicle and ventilated through shielded air slits by the natural air stream. Thus, the magnetic field was undisturbed and the birds could breathe the natural outside air during the outward journey (a condition related to findings of Papi *et al.*^{3,7}). After arriving at the release site, the basket with the experimental birds was taken out of the dark box and placed on the ground. We used two types of control: in releases 1 and 2, the control birds were transported in the wooden basket like the control birds in the first experiment, but were placed in the dark box for 30 min after arrival at the release site (Fig. 2a, open symbols). In releases 3 and 4, the controls were also transported in a similar daylight-tight wooden box on the roof of the vehicle but two small battery-operated light bulbs provided a dim light (3.4 lx) inside the box (Fig. 2a, solid symbols). The data for all four tests are given in Table 1. The control birds were significantly oriented, whereas the birds transported in total darkness showed random behaviour. These data are summarized in Fig. 2; the difference in scatter is significant ($P < 0.01$).

The control results indicate that this disorientation was not caused by simply staying in total darkness for a certain amount of time, nor by being transported visually isolated from the outside. Transportation in total darkness, as in a distorted magnetic field, seemed to make the gathering of necessary information during the outward journey impossible. (In release

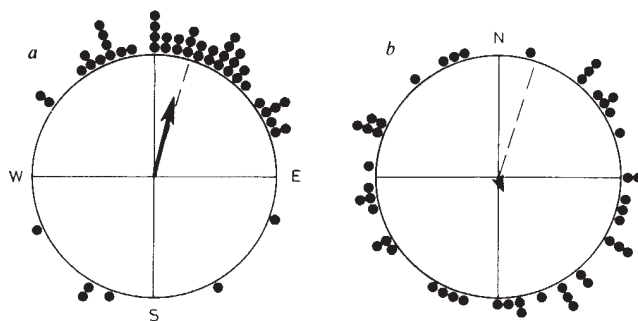


Fig. 1 Summary of the four tests demonstrating the effect of a distorted magnetic field during the outward journey. *a*, The normally transported controls were significantly oriented: $n = 50$, $\alpha = 13^\circ$, $r = 0.66$ ($P < 0.001$, Rayleigh test). *b*, The birds transported in the experimentally altered magnetic field did not show any directional preference: $n = 48$, $\alpha = 156^\circ$, $r = 0.13$ ($P \gg 0.05$). The departure bearings are represented by the symbols at the periphery of the circles; the mean vectors are given as arrows with the length r drawn proportional to the radius of the circle = 1. The home direction, 17° NNE, is marked by a dashed line.

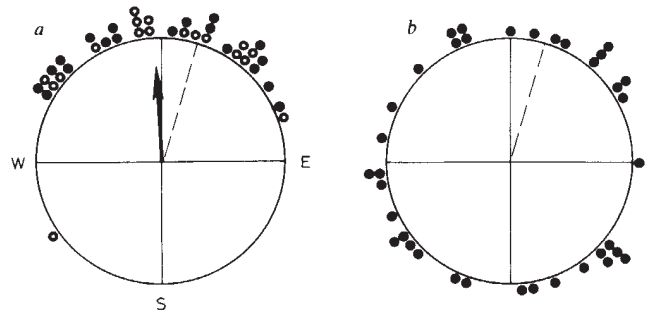


Fig. 2 Summary of the four tests demonstrating the effect of total darkness during the outward journey. *a*, Both types of control (see text) were significantly oriented: $n = 37$, $\alpha = 357^\circ$, $r = 0.76$ ($P < 0.001$). *b*, The birds transported in total darkness showed random behaviour: $n = 37$, $\alpha = 137^\circ$, $r = 0.01$ ($P \gg 0.05$). Symbols as in Fig. 1.

4 we controlled against psychological effects by habituating the designated experimental birds to being driven around in the dark box before the critical test, but this did not seem to help their orientation.)

Our findings are consistent with a major prediction derived from the optical resonance hypothesis. But, as 'negative results', they are open to various explanations, since we do not know the exact cause of the disoriented behaviour of our experimental birds. 'Orientation' as recorded in homing experiments with birds is a highly complex behaviour, the observed disorientation shows that one of the functions leading to the behavioural output had been disrupted, but we cannot tell at what level, nor can we discriminate between an effect on the magnetic receptor and other effects (like, for example, the birds falling asleep or feeling sick when transported in darkness). This dilemma must be considered when our findings are discussed in the light of Leask's¹ hypothesis, the more, since other orientation experiments with caged migratory birds have yielded ambiguous results (unpublished).

Had the outcome of our experiments been reversed, that is, had transportation in total darkness not affected the birds' orientation, it would have clearly shown that Leask's¹ model is incorrect, at least for magnetoreception in birds. The results, as they turned out, do not allow a definite conclusion about the model: they suggest that it might apply, but other specific or nonspecific interferences with the orientation behaviour are equally possible.

Nonetheless, transportation in total darkness is a methodically simple way of preventing pigeons from obtaining orientation information during the outward journey, and this will help any future study of the significance of route-specific information compared with site-specific information in the orientation system of birds⁹.

This work was supported by the Deutsche Forschungsgemeinschaft, all calculations were carried out by the Hochschulrechenzentrum of the Universität Frankfurt aM. We thank our helpers, especially E. Franke, H. Golle, M. Grüter and V. Reichard, for assistance with the releases, and M. J. M. Leask for critically reading an early draft of this manuscript.

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1. Leask, M. J. M. *Nature* **267**, 144–145 (1977).
2. Wiltschko, R. & Wiltschko, W. *Science* **176**, 62–64 (1972).
3. Papi, F., Ioalè, P., Fiaschi, V., Benvenuti, S. & Baldaccini, N. E. *Animal Migration, Navigation and Homing* (eds Schmidt-Koenig, K. & Keeton, W. T.) 65–77 (Springer, Berlin, 1978).
4. Kiepenheuer, J. *Naturwissenschaften* **65**, 113 (1978).
5. Wiltschko, R. & Wiltschko, W. *Naturwissenschaften* **65**, 112–113 (1978).
6. Wiltschko, R., Wiltschko, W. & Keeton, W. T. *Animal Migration, Navigation and Homing* (eds Schmidt-Koenig, K. & Keeton, W. T.) 152–162 (Springer, Berlin, 1978).
7. Papi, F. *Verh. dt. zool. Ges.* **1976**, 184–205 (1976).
8. Batschelet, E. *Statistical Methods for the Analysis of Problems in Animal Orientation and Certain Biological Rhythms* (AIBS, Washington DC, 1965); *Animal Orientation and Navigation* (eds Galler, S.R. *et al.*) 61–63 (NASA SP-262, Washington DC, 1972).
9. Wiltschko, W. & Wiltschko, R. *Oikos* **30**, 177–187 (1978).

BOOK REVIEWS

Meeting of physics and metaphysics

Abner Shimony

THIS book is David Bohm's attempt to effect a meeting of East and West. In the basic principles of his metaphysics and epistemology, the East predominates. The world is an undivided whole, and any analysis of it into parts (things, atoms, sensations) is an erroneous fragmentation. Unlike the static monism of Parmenides, the monism of Bohm emphasizes process: "all is flux" (p.48). A primary instance of violating the wholeness of the world is the dichotomy of mind and matter. It is incorrect to regard knowledge as a correspondence between thoughts and things, because both of these termini of the hypothetical correspondence relation are abstracted from the total process. Bohm proposes instead "the notion that thought is a sort of 'dance of the mind' which functions indicatively" (p.55). Ordinary language with its characteristic subject-verb-object structure is inadequate both to the holistic and the process character of reality. Consequently, Bohm proposes a new mode of discourse called "the rheomode", from the Greek word meaning "to flow", in which the verb rather than the noun is given a primary role.

Bohm acknowledges, however, that the West has something to contribute to wisdom, since "measure is insight into a secondary and dependent but nevertheless necessary aspect of reality" (p.23). Indeed, if it were not for his effort to relate modern physics to his metaphysics there would be little reason to single this book out for attention from among numerous variant versions of holistic metaphysics. One might be content with the succinct exposition of Enoch Soames: "Life is web, and therein nor warp nor woof is, but web only" (in Max Beerbohm's *Seven Men*; Heinemann, 1919).

Over three decades Bohm has made distinguished contributions to the foundations of quantum mechanics. In the present book his position regarding quantum mechanics is a complicated synthesis of his previous ideas. He continues to search for a theory of hidden variables on a sub-quantum level. He recognizes, however, that in situations of the type considered by Einstein, Podolsky and Rosen, in which a non-factorizable wave function is used to describe a pair of spatially separated particles, no *local* hidden variables theory is capable of agreeing with all the experimental predictions of quantum mechanics. (The argument which

Wholeness and the Implicate Order. By David Bohm. Pp.224. ISBN 0-7100-0366-8. (Routledge & Kegan Paul: 1980.) £8.95, \$25.

he gives to this effect on pp.72-73 is unfortunately very elliptical and could not be followed by any reader who is not acquainted with the work on local hidden variables theories of J.S. Bell and his followers.) Bohm is willing, however, to accept non-local hidden variables theories. He thus seems to believe that some of the features of present-day quantum theory — especially holism and non-locality — will remain embedded permanently in the *Weltanschauung* of physics even after the development of a sub-quantum physics; indeed, two of his seven chapters contain "Quantum theory as an indication of a new order in physics" as part of their titles. Clearly, the features of quantum mechanics which he expects to be perpetuated are precisely those which mesh with his metaphysics of holism and flux.

Since the causal structure of relativistic space-time appears to be violated in any non-local hidden variables theory, the following question is posed: why is it not possible to transmit signals faster than light? Bohm answers that only random motions on a sub-quantum level are superluminal, whereas the average motions of particles, which are relevant in signal propagation, are governed by relativity theory. This answer can be assessed only when the physical implications are worked out in detail and appropriate experiments are performed.

What is most puzzling about Bohm's philosophy is the problem which it shares with all varieties of holism: how does an undivided existence manage to exhibit itself in fairly well articulated parts and aspects? Bohm attempts to solve this problem by the interplay of the "implicate order" and the "explicate order" (Chapters 6 and 7). In the implicate order "the totality of existence is enfolded within each region of space and time" (p.172).

Nevertheless, . . . the overall law (holonymy) may be assumed to be such that in a certain sub-order, within the whole set of implicate order, there is totality of forms that have an approximate kind of recurrence, stability and separability . . . The special distinguished sub-order indicated above, which is the basis of the

possibility of this manifest world, is then, in effect, what is meant by the explicate order [p.186].

Bohm's favourite illustration is the hologram, in which a whole illuminated structure is mapped by Fourier optics onto every finite region of a photographic plate, with the result that the original optical display can be reconstructed (though with some loss of detail) even if all but a small part of the photographic plate is destroyed. The optical display reconstructed by interfering laser beams is the "explicate order", which makes manifest the "implicate order" in the photographic plate. Of course, this illustration is little more than a metaphor, since holography can be explained in terms of classical electromagnetic theory, which does not represent reality as an undivided whole. (Each component of the electromagnetic field at each point on a space-like surface is a separate degree of freedom, and all of these infinitely-many degrees of freedom can in principle be assigned independent values.) Nevertheless, the metaphor of the hologram is suggestive, and Bohm's conception of the implicate and explicate orders does indeed seem to provide him with an inspiring heuristic for revising physical theory.

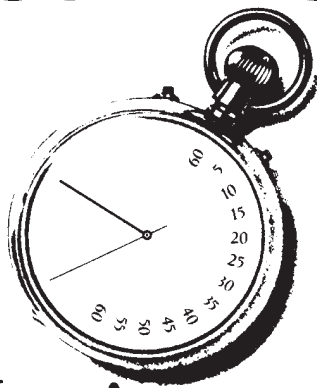
Bohm's programme is ambitious, and in the long run it must be assessed by concrete achievements. He hopes to remove the mystery from the reduction of wave packets; he wishes to eliminate the divergences which bedevil relativistic quantum field theory and to dispense with techniques such as renormalization. Since the quantization of the gravitational field suggests that it may be meaningless to speak of spatial distances less than 10^{-33} cm, he hopes to be able to throw new light upon the structure of space-time in the small. And he expects new phenomena to be observed when the "immense background of energy" in so-called empty space is properly probed. The sketch which Bohm provides of the mathematical apparatus of his programme on pp.155-171, is, unfortunately, so cryptic that I can make no judgements about its prospects. I can only say that he has achieved enough as a physicist to deserve both time for pursuing his programme and attention for his claims of concrete results.

It is appropriate to compare Bohm's book with Fritjof Capra's *The Tao of Physics* (Wildwood House, 1975), which

also advocates the holistic metaphysics of the East and links it with modern physics. Capra, incidentally, quotes approvingly from an important paper of Bohm and Basil Hiley. Capra's book is pleasant to read, for he writes fluently, quotes charming *haikus* and *Zen koans*, and even offers some beautiful photographs of sculpture and calligraphy. Bohm's style, by contrast, is ponderous and his exposition abstract. What I find unattractive in Capra's book is the way in which he draws up a brief that "the principal theories and models of modern physics lead to a view of the world which is internally consistent and in perfect harmony with the views of Eastern mysticism". He relies uncritically upon the controversial bootstrap theory of strong interactions, because it fits his metaphysics; he dissolves the precise meaning of technical terms in order to establish analogies; and he plays down discrepancies between points of view when they threaten his parallels. Bohm's book has some of the same tendencies, but to a much lesser degree. *Wholeness and the Implicate Order* conveys a sense of work in progress, which aims at a distantly glimpsed ideal of the unification of all the aspects of the world, and it is refreshingly free from claims that the ideal has already been achieved. The feeling of struggle in Bohm's book is its most appealing feature.

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IRCS JOURNALS



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Birthday party for the skinned fibre

C.R. Bagshaw

Muscle Contraction: Its Regulatory Mechanisms. Edited by S. Ebashi *et al.* Pp. 549. ISBN 3-540-10411-9/0-387-10411-9. (Japan Scientific Societies Press, Tokyo/Springer-Verlag: 1980.) DM108, \$63.80.

DURING October 1979 an international symposium on muscle contraction was held near Mount Fuji in honour of two eminent Japanese scientists, Professor Hiroshi Kumagi and Professor Reiji Natori. In 1955 Kumagi and colleagues identified the "relaxing factor" of muscle as a microsomal ATPase — the now-familiar Ca^{2+} pump of the sarcoplasmic reticulum. Since then he has been a central figure in organizing and stimulating muscle research in Japan. Natori's "skinned fibre" needs little introduction, having provided a unique preparation amenable to combined physiological and biochemical manipulations. These symposium proceedings have been published in commemoration of Professor Kumagi's seventy-fifth and the skinned fibre's formal twenty-fifth birthday.

The majority of the 42 contributions comprise summaries of recent work, but without unnecessary duplication of published data. Instead, many authors have introduced the historical events and intuitions which have guided the direction of their research. A.F. Huxley starts the proceedings with a review that makes the library stacks as enlightening as the current journal shelves. He describes the initial reluctance to accept that relaxing agents work by chelating Ca^{2+} — I wonder if we have become too complacent in our use of EGTA. Natori relates how his skinned fibre helped in the evaluation of the excitation-contraction mechanism, but not content to dwell on history he goes on to describe the contribution of connectin to passive elasticity.

The remaining papers range from studies of cross-bridge movement to the cytoskeleton, but predominantly concern regulatory mechanisms. Unfortunately, while the controversies regarding smooth muscle control are outlined, insufficient data are presented to allow a rational assessment. Hartshorne and Adelstein focus on the properties of kinases, while Nonomura's leiotonin data are limited. Watanabe presents the noteworthy result that desensitized molluscan actomyosin does not superprecipitate, yet Szent-Györgyi shows that desensitized fibres contract regardless of Ca^{2+} . Over the past decade superprecipitation has fallen out of favour as a quantitative probe; clearly, caution is required even in its qualitative applications, particularly in preparations which may possess atypical solubility at low ionic strength.

This volume has achieved its aim of bringing together current research on

regulatory mechanisms. The readable styles adopted by most authors serve to complement the existing literature, and the editors are to be congratulated on compiling a fitting and useful tribute. □

C.R. Bagshaw is a Lecturer in Biochemistry at the University of Leicester.

Polymers in the lab

Herman Mark

Experimental methods in Polymer Chemistry. By J. F. Rabek. Pp. 861. ISBN 0-4712-7604-9. (Wiley: 1980.) £52.80, \$145.20.

THIS presentation of a large and important field of modern chemistry is truly remarkable, particularly since it has been compiled and written by a single author.

Two chapters are essentially descriptive, as they ought to be — the overviews of the structure (Chapter 1) and of the morphology (Chapter 26) of polymers; both contain well-selected and educationally impressive sketches and photographs of all aspects of their topics. In addition they make excellent reading. The bulk of the book is really a condensed encyclopaedia of all the basic characteristics of synthetic polymers. In each case (for instance light scattering, Chapter 13) there is first a clear yet concise presentation of the theoretical background, then a description of the best existing instruments with adequate instructions for making sample preparations and, finally, the most recent improvements and modifications of the particular method are discussed. This enumeration starts with dissolution and goes over fractionation, osmometry, ultracentrifugation, viscometry, all spectroscopic methods to the scattering of X-rays, electrons and neutrons, to NMR and the various chromatographic techniques.

Deserving of particular mention are the condensed but clear presentations of the principles of every individual technique and the many long and carefully assembled tables on significant results, for example those on solubility parameters, NMR parameters and ESR parameters. A special feature of the book, and at the time proof of the never-ending patience of the author, is the bibliography which takes up almost 150 pages and lists 7,350 entries.

If anybody wants to get — or stay — in close touch with polymer chemistry, he ought to buy this book and carry it with him all the time; there is no better source of information on the subject. □

Herman Mark is Emeritus Dean of the Polytechnic Institute of New York, and editor of the *Journal of Polymer Science*.

Henry Stommel and physical oceanography since 1945

K.F. Bowden

Evolution of Physical Oceanography. Edited by Bruce A. Warren and Carl Wunsch. Pp.623. ISBN 0-262-23104-2. (MIT Press: 1981.) \$37.50, £23.25.

THIS volume is a tribute, on the occasion of his sixtieth birthday, to Henry Stommel who has been a pre-eminent figure in the development of physical oceanography since the Second World War. He is probably best known for showing, in an early paper published in 1948, that the intensification of currents on the western side of an ocean is due to the variation of the Coriolis parameter, which expresses the effect of the Earth's rotation, with latitude. This is demonstrated clearly by the relatively narrow, fast-flowing Gulf Stream in the North Atlantic and the Kuroshio in the North Pacific Ocean, but it is a dynamical feature of deep currents as well.

IMAGE
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Henry Stommel

Stommel has subsequently made remarkable contributions to practically every aspect of physical oceanography, from mixing in estuaries to the world-wide deep circulation of the oceans. His work has been characterized by its physical insight, expressed in terms of the simplest dynamical models, which others have developed in greater mathematical detail. He has also played a leading part in the move to direct field work in oceanography away from the survey of geographical areas to the study of physical processes.

In their preface the editors explain why, instead of the traditional *Festschrift*, this volume takes the form of a series of broad, comprehensive surveys of aspects of oceanography with which Stommel has been concerned. Together they present a most valuable picture of the evolution of physical oceanography over the past 35 years and its present status. The surveys are preceded by short contributions from five former colleagues, giving an appreciation of Henry Stommel and his work.

The main body of the book is comprised of 18 chapters grouped into four parts. The first, on the general ocean circulation, is the longest. It has surveys of the deep circulation of the world ocean by B.A. Warren, on water masses by L.V. Worthington, the mid-depth circulation by J.L. Reid and the Gulf Stream System by N.P. Fofonoff. In his chapter on the dynamics of large-scale ocean circulation, G. Veronis gives a comprehensive account of the vast amount of theoretical work in this field over the past 30 years. Equatorial currents are clearly described in observation and theory by A. Leetmaa, J.P. McCreary and D.W. Moore. The chapter on estuarine and continental-shelf circulation in the Middle Atlantic Bight, by R.C. Beardsley and W.C. Boicourt, is of wider interest than its title might suggest, as the treatments and results are applicable to many other areas.

Part Two deals with physical processes in oceanography. The first chapter, by J.S. Turner, is on small-scale mixing processes and is followed by that of W. Munk on internal waves and small-scale processes. Long waves and ocean tides are reviewed by M.C. Hendershott, while C. Wunsch discusses the low-frequency variability of the sea, a feature which has long been recognized but has only recently come to be studied with a thoroughness approaching that with which meteorologists have treated the atmosphere. In the only contribution of a biological nature, J.H. Steele discusses the interaction of physical, biological and ecological processes in the distribution of organisms. W.V.R. Malkus rounds off this section with a chapter on the amplitude of convection.

The practical oceanographer will welcome Part Three, "Techniques of Investigation", which opens with a good review of instrumentation and the design of experiments by D.J. Baker. W.S. Broecker discusses geochemical tracers and ocean circulation, dealing mainly with the use of radioisotopes, particularly the data from GEOSECS (Geochemical Ocean Sections Studies). The use of laboratory models in the study of ocean circulation is described by A.J. Faller. In the final section on ocean and atmosphere, H. Charnock surveys the topic of air-sea interaction, while J.G. Charney and G.R. Flierl describe ocean analogues of large-scale atmospheric motions, concentrating mainly on transient motions.

In a volume of this kind there is bound to be a certain unevenness among the contributions, although here the treatment is more consistent than in most collective works. All chapters are of high quality and comprehensive in scope, the variations being in depth and in the amount of mathematical detail in the chapters on theoretical topics. This volume has succeeded admirably in its

purpose of paying tribute to a great oceanographer and providing a comprehensive and inspiring survey of physical oceanography today. □

K.F. Bowden is Professor of Oceanography at the University of Liverpool.

Surface tension

Peter Schofield

Statistical Mechanics of the Liquid Surface. By Clive A. Croxton. Pp.368. ISBN 0-4712-7663-4. (Wiley: 1980.) £25, \$68.75.

THE first thing to be said about this volume is that the title is wrong. The book is mainly concerned with the *molecular theory* of liquid surfaces, that is with methods of calculation of the properties of liquid-vapour interfaces either from the intermolecular forces and molecular distribution functions (the Kirkwood-Buff approach) or, to a lesser extent, from the old Van der Waal's theory and its modern counterpart in terms of the density gradients and density fluctuations (the Yvon-Triezenberg-Zwanzig approach). In both cases, the derivation from statistical mechanics is given in a manner which the uninitiated reader will find difficult to follow.

The fundamental question about surface tension is whether indeed it has an intrinsic value for a given fluid. The width of the interface is determined by the amplitude of thermal capillary wave fluctuations, which is unbounded in the limit of infinite volume and zero gravity since there is then no cost in free energy in a rotation of the whole system. It is on this quicksand that the molecular theory is built!

Nevertheless, considerable success has been achieved in predicting the properties of the liquid-vapour interface by ignoring such problems, and Clive Croxton has done a service in bringing together this review, with a comprehensive bibliography, of calculational methods for several types of fluid. Following two introductory chapters, the subjects include the surfaces of molecular, metallic and quantum liquids, liquid crystals and polymer adsorption at solvent surfaces. The question of molecular orientation in an interface and surface potentials is of considerable practical importance, and in this respect there is a good chapter on water.

As in many fields of physics and physical chemistry, computer simulation methods have a major role to play in bridging the gap between theory and experiment. On

V. Cullen

the evidence of the chapter devoted to this topic, these techniques were only just approaching the stage of giving definitive results for the liquid surface at the time of writing, though progress has been made since. The situation resembles that for bulk liquids some 20 years ago. There is no doubt that computer simulation can likewise transform our understanding of the molecular properties of surfaces.

Computer simulation, however, will not solve the deeper statistical mechanics problems of co-existing phases of real fluids, such as the conditions for phase separation and critical behaviour. For these, one needs the full, modern apparatus of renormalization group and nucleation theory, but such considerations are outside the scope of this book. □

Peter Schofield is a Group Leader in the Materials Physics Division at Harwell, and has overall responsibility for the Neutron Beam Research Programmes of the Atomic Energy Authority.

The iris as history

J.C. Dean Hart

The Iris in Eighteenth-century Physiology. By Renato G. Mazzolini. Pp.193. ISBN 3-456-81022-9. (Hans Huber, Bern: 1980.) Flexi SwFr. 36, DM 39.

RECENT reviews of the historical aspects of iris musculature, notably that of Loewenfeld 1957, when dealing with the eighteenth century use as their first definitive reference the report by Albrecht von Haller, Professor of Anatomy at Göttingen, who in 1753 stated that the iris did not contain muscles. This opinion flatly contradicted the contemporary view that an antagonistic set of muscles was present in the iris, one for constriction of the pupil, the other for dilation. However, no recent author has made mention of the persons or person responsible for initiating the muscular concept or of how it became widely disseminated. Here, in an erudite piece of historical investigation, Mazzolini has tracked down the source of the muscular theory and convincingly demonstrated reasons as to why it became so readily accepted by most authorities on the Continent and as far away as Scotland. He has set out to provide an historical analysis of the statement by Haller that "all muscles are irritable — the iris has no irritability", and has attempted to answer three questions: was this remark original; to what extent did Haller's comments alter views of students on the subject; and how did this change previously held ideas? It would, however, be unfair in this review to reveal all the details of Mazzolini's research, as his is an excellent piece of detection.

Haller began to have serious doubts about the presence of muscles in the iris as early as 1743, since, unlike earlier researchers, he was quite unable to see any such structures. In 1753, his major work was published, *De partibus corporis humani sensilibus et irritabilibus*, in which an entirely new concept of physiological thinking was set out based solely on the twin pillars of experimentation and observation. His novel proposal was that the body could be divided into three types of tissue depending on how they reacted to cutting, burning or the application of noxious fluids: those showing irritability (contractability) were muscles; those demonstrating sensibility, nerves; the remainder, having neither of these attributes, were called *tela cellularis*.

This work proved to be a landmark since it introduced a radical alternative to the earlier scientific approach based on the Galenic tradition of analogy and reason. The case for iris muscles had been argued previously in the form of a syllogism as follows: structures that contain muscles contract; the iris contracts; therefore this tissue contains muscles. Haller, however, believed that analogy was a source of great error and that it was not necessary to devise fabrics which could not be observed. Since irritability of the iris was not detectable in experimental animals when this tissue was cut or after the application of oil of vitreol — and supported by his earlier observation that muscles could not be seen — he felt that the idea of a muscular mechanism of iris movement had to be abandoned. True to the tradition that he had created, Haller proposed instead that the blood vessels of the iris, which could be readily identified using wax injection techniques, became dilated or constricted, thus permitting blood to flow in and out and inducing movements similar to the type known to occur in the erection of the penis. The erectile theory continued to be predominant for the next 70 years or so; only when improved microscopes became available and tissues could be sectioned and suitably stained, in the 1820s, was the sphincter muscle finally identified beyond all reasonable doubt. In the case of the dilator muscle, however, a fierce controversy was only quelled in the early part of the twentieth century when embryological studies revealed that both the dilator and constrictor muscles were derived from neural ectoderm.

In this book, Mazzolini has accomplished the task which he set himself; Haller's hypothesis does appear to have been original and to have radically influenced contemporary views. However, there are a few aspects in the text where further elaboration might have been helpful. Mazzolini has attempted by experimentation and using techniques similar to those employed by eighteenth century anatomists to determine how a number of ideas concerning iris movements were derived, but he has not sought

to question the truth of Haller's statement — "the iris is not irritable". This is unfortunate as one gains the impression that Haller's concept remains correct. Modern ophthalmologists would agree that in the Hallerian sense the human iris does in fact exhibit irritability, since if it is accidentally photocoagulated or subjected to excessive manipulation — for example, during cataract surgery — changes in the size of the pupil may occur, albeit quite slowly.

Also, throughout the book, philosophical concepts prevailing during the eighteenth century are discussed which the author believes may have had an influence on attitudes on various workers on reaching their conclusions. But, particularly in the last chapter on *Naturphilosophen*, such theories have not been sufficiently well described for anyone who is not well versed in continental ideologies of the period to achieve an adequate understanding of a number of rather complex propositions. It likewise seems unnecessary to contemplate whether the Scotsman, Robert Whytt (1714–1766) was an unorthodox Stahlian or not, irrespective of Haller's views on this subject, since his reasoning on the nature of the pupillary responses to alterations of illumination is of the very highest quality in his work *An essay on the vital and other involuntary movements of animals*, published in 1751. The fact that he considered the soul or sentient spirit developed an awareness that the retina had become uneasy if too much light impinged upon it and responded by causing pupillary constriction, could be explained equally well on the basis that Whytt, in trying to describe the concept of a reflex, might have been attempting to avoid rousing the animosity of the powerful national religious orthodoxy of the period. Whytt's views were temporarily discredited, not so much because he believed in animism, but because there was insufficient experimental data to support his supposition in terms of Haller's new physiology.

The eighteenth century was a period of profound importance in the development of the medical sciences; it was during this time that the use of experimentation became widely respectable and the mists of archaic speculation began to melt away. Mazzolini is to be congratulated on providing a clear and eminently readable exposition of a period of the history of the iris which has recently suffered almost total neglect. Although speculations relating to the mechanics of the iris might appear to be a somewhat esoteric subject, we are given a clear indication of the wider aspects of physiological investigations that were being pursued during this period, which included the first steps as to how the fundus might be examined *in vivo* and the role of the retina as a light-sensitive tissue. □

J.C. Dean Hart is Head of the Department of Ophthalmology at the University of Bristol.

BOOKS RECEIVED

Mathematics

- ADLER, R. J. *The Geometry of Random Fields*. Pp.280. ISBN 0-471-27844-0. (Wiley: 1981.) £17.50.
- BOYCE, W. E. (ed.). *Case Studies in Mathematical Modeling*. Pp.386. ISBN 0-273-08486-0 (Pitman Publishing: 1981.) £22.
- DRAZIN, P. G. and REID, W. H. *Hydronamic Stability*. Cambridge Monographs on Mechanics and Applied Mathematics. Pp.525. ISBN 0-521-22798-4. (Cambridge University Press: 1981.) £35.
- LAPWOOD, E. R. and USAMI, T. *Free Oscillations of the Earth*. Pp.243. ISBN 0-521-23563-7. (Cambridge University Press: 1981.) £25.
- REIN, W. T. *Sturmian Theory for Ordinary Differential Equations*. Applied Mathematical Sciences, Vol.31. Pp.559. Flexi ISBN 3-540-90542-1. (Springer-Verlag: 1980.) DM 54, \$31.90.

Physics

- BOUMANS, P. W. J. M. *Line Coincidence Tables for Inductively Coupled Plasma Atomic Emission Spectrometry*. 2 Vol. set. Pp.951. ISBN 0-08-026243-0-H (Pergamon: 1980.) £104, \$250.
- BUBE, R. H. *Electrons in Solids. An Introductory Survey*. Pp.229. ISBN 0-12-138650-3. (Academic: 1980.) \$25.
- FRAISSARD, J. P. and RESING, H. A. (eds). *Magnetic Resonance in Colloid and Interface Science*. NATO Advanced Study Institutes Series. C. Mathematical and Physical Sciences, 61. Proceedings of a NATO Advanced Study Institute and the 2nd International Symposium held at Menton, France, June 1979. Pp.710. ISBN 90-277-1153-4. (Reidel: 1980.) Dfl.145, \$76.
- GARRIDO, L. (ed.). *Systems Far from Equilibrium*. Lecture Notes in Physics, 132. Sitges Conference on Statistical Mechanics, June 1980, Sitges, Barcelona, Spain. Pp.403. Flexi ISBN 3-540-10251-5. (Springer-Verlag: 1980.) DM147, \$27.80.
- LAWRENCE, J. H. GOFMAN, J. W. and HAYES, T. L. (eds.) *Advance in Biological and Medical Physics*, Vol. 17. Pp.473. ISBN 0-12-005217-2. (Academic: 1980.) \$55.
- PAMPLIN, B. R. (ed.). *Crystal Growth*. 2nd Edn. Pp.609. ISBN 0-08-025043-2. (Pergamon: 1980.) £60, \$140.
- WOLF, K. B. (ed.). *Group Theoretical Methods in Physics*. Lecture Notes in Physics, 135. Proceedings of the IX International Colloquium held at Cocoyoc, Mexico, June 1980. Pp.629. Flexi ISBN 3-540-10271-X. (Springer-Verlag: 1980.) DM72 \$42.50.

Chemistry

- HANNA, L. W. *Quantum Mechanics in Chemistry*. 3rd Edn. Pp.284. Hbk ISBN 0-8053-3708-3; pbk ISBN 0-8053-3705-9. (Benjamin/Cummings: 1981.) Hbk \$14.95; pbk \$9.95.
- ORCHIN, M. *et al.* *The Vocabulary of Organic Chemistry*. Pp.609. ISBN 0-471-04491-1. (Wiley: 1981.) £18.75, \$43.75.
- TEJA, A. S. (ed.). *Chemical Engineering and the Environment*. Critical Reports of Applied Chemistry, Vol.3. Pp.100. Flexi ISBN 0-632-00693-5. (Blackwell Scientific: 1981.) £8.

Technology

- COUNT, B. (ed.). *Power from Sea Waves*. Based on the Proceedings of a Conference held at University of Edinburgh from 1979. Pp.450. ISBN 0-12-193550-7. (Academic: 1980.) £23.60, \$57.
- JENKINS, S. H. (ed.). *Mediterranean Coastal Pollution*. Progress in Water Technology. Proceedings of a Conference held in Palma, Mallorca, September 1979. Pp.870. ISBN 0-08-026058-6. (Pergamon: 1980.) £44.50, \$100.
- KUO, F. F. *Protocols and Techniques for Data Communication Networks*. Pp.468. ISBN 0-13-731729-8. (Prentice-Hall: 1981.) £19.45
- OLSZEWSKI, M. (ed.). *Utilization of Reject Heat*. Energy Power and Environment, Vol.9. Pp.208. ISBN 0-8247-1168-8. (Marcel Dekker: 1980.) SwFr.65.
- SOCIETE FRANCAISE D'ENERGIE NUCLEAIRE. *Colloquium on the Risks of Different Energy Sources*. Pp.658. (Gedim, 19 Rue du Grand-Moulin, 42029 Saint-Etienne Cédex: 1980.) Flexi np.

Earth Sciences

- GUPTA, H. K. *Geothermal Resources: an Energy Alternative*. Developments in Economic Geology, 12. Pp.220. ISBN 0-444-41865-2. (Elsevier Scientific: 1980.) \$61, Dfl.125.
- LYNCH, A. J. *et al.* *Mineral and Coal Flotation Circuits*. Their Simulation and Control. Developments in Mineral Processing, 3. Pp.291. ISBN 0-444-41919-5. (Elsevier Scientific: 1981.) \$63.50, Dfl.130.
- NEELY, W. B. *Chemicals in the Environment*. Distribution, Transport, Fate, Analysis. Pollution Engineering and Technology, Vol. 13. Pp.245. ISBN 0-8247-6975-9. (Marcel Dekker: 1980.) SwFr.82.
- STRANGWAY, D. W. *The Continental Crust and its Mineral Deposits*. The Geological Association of Canada Special Paper, 20. Proceedings of a Symposium in honour of J. Tuzo Wilson, Toronto, May 1979. Pp.804. ISBN 0-919216-16-1. (The Geological Association of Canada, University of Waterloo, Waterloo, Ontario, Canada: 1980.) Np.

- WHITELEY, R. J. (ed.). *Geophysical Case Study of the Woodlawn Orebody*, New South Wales, Australia: The First Publication of Methods and Techniques Tested Over a Base Metal Orebody of the Type Which Yields the Highest Rate of Return on Mining Investment with Modest Capital Requirements. Pp.610. ISBN 0-08-023996-X. (Pergamon: 1980.) £35.50.

Biological Sciences

- ALBANESE, A. A. *Nutrition for the Elderly*. Current Topics in Nutrition and Disease, Vol.3. Pp.332. ISBN 0-8451-1602-9. (Alan R. Liss: 1980.) \$38, Dfl.114.
- ALBERTS, B. (ed.). *Mechanistic Studies of DNA Replication and Genetic Recombination*. ICN - UCLA Symposia on Molecular and Cellular Biology, Vol. 19, 1980. Pp.1003. ISBN 0-12-048850-7. (Academic: 1980.) \$48.
- BALTES, P. B. and BRIM, O. G. JR. (eds). *Life-Span Development and Behaviour*, Vol.3. Pp.412. ISBN 0-12-431803-7. (Academic: 1980.) \$35
- BURKE, S. R. *The Composition and Function of Body Fluids*. 3rd Edn. Pp.208 Pbk. ISBN 0-8016-0903-8. (C. V. Mosby: 1980.) Pbk np.
- COHN, W. E. (ed.). *Progress in Nucleic Acid Research and Molecular Biology*, Vol.24. Pp.279. ISBN 0-12-540024-1. (Academic: 1980.) \$42.50.
- EDHOLM, O. G. and WEINER, J. S. (eds). *The Principles and Practice of Human Physiology*. Pp.672. ISBN 0-12-231650. (Academic: 1981.) £36.80, \$88.50.
- EISENBERG, J. M. *A Collector's Guide to Seashells of the World*. Pp.239 ISBN 0-07-019140-9. (McGraw-Hill: 1981.) £12.50.
- ERIKSSON, A. W. *et al.* (eds). *Population Structure and Genetic Disorders*. Sigrid Jusélius Foundation Symposium, Mariehamn, Åland Islands, Finland, August 1978. Pp.690. ISBN 0-12-241450-0. (Academic: 1980.) £55, \$126.50.
- FREEDMAN, R. B. and HAWKINS, H. C. *The Enzymology of Post-translational Modification of Proteins*. Vol.1, Molecular Biology. Pp.515. ISBN 0-12-266501-5. (Academic: 1980.) £40, \$92.
- GÖRLIN, R. (ed.). *Morphogenesis and Malformation of the Ear*. Birth Defects: Original Article Series 1980. Vol.16. No.4. Fifth International Workshop held at Gulf State Park, Gulf Shores, Alabama. Pp.367. ISBN 0-8451-1038-1. (Alan R. Liss: 1980.) \$44, Dfl.132.
- HANCE, R. J. (ed.). *Interactions Between Herbicides and the Soil*. Pp.350. ISBN 0-12-323840-4. (Academic: 1980.) £20.60, \$49.50.
- HUNT, R. K. *Neural Development*, Part II. *Neural Development in Model Systems*. Current Topics in Developmental Biology, Vol.16. Pp.410. ISBN 0-12-153116-3. (Academic: 1980.) \$34.
- KANEKO, J. J. (ed.). *Clinical Biochemistry of Domestic Animals*. 3rd Edn. Pp.832. ISBN 0-12-396350-8. (Academic: 1980.) £60.
- MATTA, M. S. and WILBRAHAM, A. C. *Atoms, Molecules and Life: An Introduction to General Organic and Biological Chemistry*. Pp.721. ISBN 0-8053-9640-3. (Benjamin/Cummings: 1981.) Np.
- PHILLIPSON, J. D. and ZENK, M. H. (eds). *Indole and Biogenetically Related Alkaloids*. Annual Proceedings of the Phytochemical Society of Europe, No.17. Pp.380. ISBN 0-12-554450. (Academic: 1980.) £32.60, \$75.
- PRICE, J. H. IRVINE, D. E. G. and FARNHAM, W. F. (eds). *The Shore Environment*. Vol.1, Methods, Vol.2, Ecosystems. The Systematics Association Special Volume, No.17 (a). Proceedings of an International Symposium held at Portsmouth Polytechnic. Vol.1, Pp.321, ISBN 0-12-564701-8; Vol.2, Pp.722, ISBN 0-12-564702-6. (Academic: 1980.) Vol.1 £24.80, \$57.50; Vol.2 £48.20, \$111.
- ROTHMAN, H. *et al.* (eds). *Biotechnology. A Review and Annotated Bibliography*. Pp.141. ISBN 0-903804-72-7. (Frances Pinter, London: 1981.) £11.
- ROTTER, F. L., SAMLOFF, I. M. and RIMOIN, D. L. (eds). *Genetics and Heterogeneity of Common Gastrointestinal Disorders*. Proceedings of an International Workshop held in Indian Wells, California, March 1980. Pp.582. ISBN 0-12-598760-9 (Academic: 1980.) \$35.
- SACKNER, M. A. *Diagnostic Techniques in Pulmonary Disease*. Lung Biology in Health and Disease, Vol.16. Pp.537. ISBN 0-8247-1059-2. (Marcel Dekker: 1980.) SwFr.108.
- SCHROEDER, H. E. *Differentiation of Human Oral Stratified Epithelia*. Pp.308. ISBN 3-8055-1462-X. (Karger, Basel: 1981.) SwFr.159. DM190, \$95.25.
- SOWERS, J. R. (ed.). *Hypothalamic Hormones*. Benchmark Papers in Human Physiology, 14. Pp.342. ISBN 0-87933-358-8. (Dowden, Hutchinson & Ross: 1980.) \$40. (Distributed by Academic Press.)
- STEFFEN, C. and LUDWIG, H. (eds). *Clinical Immunology and Allergology*. Developments in Immunology, Vol.14. Proceedings of the Symposia at the XIth Congress of the European Academy of Allergy and Clinical Immunology held in Vienna, Austria, October 1980. Pp.420. ISBN 0-444-80312-2. (Elsevier/North-Holland Biomedical: 1981.) \$63, Dfl.129.
- THODY, A. J. *The MSH Peptides*. Pp.162. ISBN 0-12-687850-1. (Academic: 1981.) £16, \$38.50.
- WYNDER, E. L. (Conference Chairman). *Plasma Lipids: Optimal Levels for Health*. Proceedings of the Conference on Health Effects of Blood Lipids: Optimal Distributions for Populations Held by the American Health Foundation, New York, April 1979. Pp.187. ISBN 0-12-103450-X. (Academic: 1980.) \$15.
- YUDEM, M. B. H. and PAYKEL, E. S. (eds). *Monoamine Oxidase Inhibitors*. The State of the Art. Pp.214. ISBN 0-471-27880-7. (Wiley: 1981.) £13.
- ZENZ, C. (ed.). *Developments in Occupational Medicine*. Pp.477. ISBN 0-8151-9862-0. (Year Book Medical Publishers: 1980.) Np.
- ZLOTIN, R. I. and KHODASHOVA, K. S. *The Role of Animals in Biological Cycling of Forest - Steppe Ecosystems*. Pp.221. ISBN 0-87933-377-4. (Dowden, Hutchinson & Ross: 1980.) \$19.50. (Distributed by Academic Press.)

ANNOUNCEMENTS

Meetings

- 15-16 June, **Safety and Loss Prevention**, York (Mrs G.M. Nelson, IChemE, George E. Davis Building, 165-171 Railway Terrace, Rugby, UK).
- 16-17 June, **The Immune System**, Basel (Basel Institute for Immunology, Hochhaus, Bau 52, Switzerland).
- 16-18 June, **Space Shuttle**, Wembley (The Institute of Measurement and Control, 20 Peel St, London W8, UK).
- 18-19 June, **Chromatography in Biochemistry, Medicine and Environmental Research and Mass Spectrometry in Biochemistry, Medicine and Environmental Research**, Venice (Dr A. Frigerio, Via Eritrea 62, 20157 Milano, Italy).
- 23-24 June, **Food and Health — a Perspective for the 80's**, London (The British Nutrition Foundation, 15 Belgrave Square, London SW1, UK).
- 24-25 June, **Biotechnology: A Briefing for Scottish Industry**, Strathclyde, (Dr J. Aitchison, University of Strathclyde, Royal College, 204 George St, Glasgow, UK).
- 22-25 June, **Erosion and Sediment Transport Measurement**, Florence (Dr P. Tacconi, Istituto de Ingegneria Civile, Via S. Marta 3, 50139 Firenze, Italy).
- 25-26 June, **Genetic Engineering in Food and Agriculture**, Virginia (R. Nash, The Energy Bureau Inc, 41 East 42nd St, New York, New York 10017, USA).
- 30 June-10 July, **Mechanical and Thermal Behaviour of Metallic Materials**, Varenna (Societa Italiana di Fisica, Via L. Degli Andalo, 2, 40124 Bogna, Italy).
- 2 July, **Health Hazards of VDUs?**, Loughborough (B. Pierce, HUSAT Research Group, Dept of Human Sciences, Loughborough University of Technology, Leicestershire, UK).
- 3 July, **Developments in Current Polymer Research**, Aberdeen (Prof. P. Meares, Dept of Chemistry, University of Aberdeen, Meston Walk, Old Aberdeen, UK).
- 6-10 July, **An Introductory Course in Tropical Hygiene**, London (Ross Institute of Tropical Hygiene, London School of Hygiene and Tropical Medicine, Keppel St, London WC1, UK).
- 6-10 July, **Mixing in the Process Industries**, Bradford (Dr N. Harnby, Dept of Chemical Engineering, University of Bradford, West Yorkshire, UK).
- 6-10 July, **Process Safety—Theory and Practice**, Durham (Mrs M. Findlay, Dept of Chemical Engineering, Teesside Polytechnic, Borough Rd, Middlesbrough, Cleveland, UK).
- 7-9 July, **Light Microscopy '81**, London (Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford, UK).
- 29 June-10 July, **Summer School in Health Physics**, London (Dr H.D. Evans, Imperial College, London SW7, UK).
- 9-10 July, **Cost Savings in Distillation**, Leeds (Prof. G.G. Haselden, Dept of Chemical Engineering, University of Leeds, Leeds, UK).
- 13-16 July, **The Technology of Fibre-reinforced Plastics Composite Materials**, Guildford (Mr M.G. Bader, Dept of Metallurgy and Materials Technology, University of Surrey, Guildford, UK).
- 13-18 July, **Applied Rock Mechanics**, Davis (Garrett Jones, University Extension, University of California, Davis, California 95616, USA).
- 14-16 July, **Inter/Micro — 81**, Cambridge (McCrone Research Institute Ltd, 2 McCrone Mews, Belsize Lane, London NW3, UK).
- 14-24 July, **Positrons in Solids**, Varenna (Societa Italiana di Fisica, Via L. Degli Andalo, 40124 Bologna, Italy).
- 16-17 July, **Breeding Legumes for Nutritional Value**, Southampton (Nutrition Society, Dr M.I. Gurr, Shinfield, Reading, UK).
- 19-24 July, **Chemistry of Platinum Group Metals**, Bristol (Dr J.F. Gibson, The Royal Society of Chemistry, Burlington House, London W1, UK).
- 21-23 July, **Synthesis in Organic Chemistry**, Oxford (Dr J.F. Gibson, The Royal Society of Chemistry, Burlington House, London W1, UK).
- 22-24 July, **HPLC Beginners Course**, Cheshire (HPLC Technology Ltd, 4 Charlotte St, West Macclesfield, Cheshire, UK).
- 28 July-2 August, **Data Acquisition in High Energy Physics**, Varenna (Societa Italiana di Fisica, Via L. Delgi Andalò, 2, 40124 Bologna, Italy).
- 17-21 August, **Earth Tides**, New York (Prof. J.T. Kuo, 828 S.W. Mudd, Columbia University, New York, New York 10027, USA).
- 17-21 August, **Soil Morphology**, London (Dr P. Bullock, Rothamsted Experimental Research Station, Harpenden, Herts, UK).
- 23-28 August, **Solar World Forum**, Brighton (UK-ISES, 19 Albemarle St, London W1, UK).
- 28 August-1 September, **9th International Congress of ISDB**, Basel (A.A. Moscona, University of Chicago, Dept of Biology, Chicago, Illinois 60637, USA).
- 28 August-9 September, **Arc Volcanism**, Tokyo (Prof. D. Shimozuro, Earthquake Research Institute, University of Tokyo, Bunkyo-Ku, Tokyo 113, Japan).
- 30 August-4 September, **Regulatory Peptides: Functional and Pharmacological Aspects**, Brescia (M. Trabucchi, Dept of Pharmacology and Therapeutics, Via Valsabbina, 19-25100 Brescia, Italy).
- 30 August-4 September, **Optics in Biomedical Sciences**, Graz (Medizinische Ausstellungs und Werbegesellschaft, M. Rodler & Co. A-1014 Wien I, Freyung 6, Postfach 155, Austria).
- 31 August-4 September, **BAAS**, York (BA, Fortress House, 23 Savile Row, London W1, UK).
- 1-5 September, **Central Nervous System Regeneration**, Catania (Dr A.M. Giuffrida Stella, Istituto di Chimica Biologica, Università di Catania, Viale Andrea Doria, 6, 95125 Catania, Italy).
- 2-4 September, **Environment and Safety**, Wembley (IE & S Exhibition and Conferences, Newgate, Sandpit Lane, St Albans, Herts, UK).
- 31 August — 11 September, **Eighth UICC Training Course in Cancer Research**, Heidelberg (Prof Dr G. Sauer, German Cancer Research Center, Im Neuenheimer Feld, 6900 Heidelberg, FRG).
- 1-4 September, **2nd Australian National Laser Conference**, Canberra (The Secretary, 2nd Australian National Laser Conference, Dept of Engineering Physics, Research School of Physical Sciences, PO Box 4, Canberra, ACT, 2600, Australia).
- 2-4 September, **Modern Aspects of Phase Equilibria**, Teesside (Mrs M. Findlay, Dept of Chemical Engineering, Teesside Polytechnic, Borough Rd, Middlesbrough, Cleveland, UK).
- 5-10 September, **Precision and Speed in Close Range Photogrammetry**, York (K.B. Atkinson, Dept of Photogrammetry and Surveying, University College London, Gower St, London WC1, UK).
- 7-11 September, **Particle Size Measurement**, Bradford (Secretary, School of Powder Technology, University of Bradford, Bradford, West Yorkshire, UK).
- 7-11 September, **Optics in Biomedical Sciences**, Graz (Medizinische Ausstellungs- und Werbegesellschaft, M. Rodler & Co. A-1014 Wien I, Freyung 6 Postfach 155, Austria).
- 7-11 September, **Modelling and Simulation of Dynamical Chemical Engineering Processes**, Bradford (Dr J. Ingham, School of Chemical Engineering, University of Bradford, Bradford, West Yorkshire, UK).
- 8-11 September, **Down to Earth Management**, Winnipeg (7th Canadian Symposium on Remote Sensing, PO Box 1106, Winnipeg, Manitoba, Canada R3C 2X4).
- 10-11 September, **Molecular Beams in Chemical Physics**, Birmingham (Dr A.R. Burgess, Dept of Chemical and Biochemical Engineering, University College London, Torrington Place, London WC1, UK).
- 13-18 September, **15th International Congress of the International Society for Chronobiology**, Minneapolis (ISC XV Conference, College of Pharmacy, 5-110 HS Unit f, 308 Harvard St SE, Mpls, Minnesota 55455, USA).
- 17-20 September, **British Veterinary Association's Annual Congress**, Exeter (BVA, 7 Mansfield St, London W1, UK).